CHARACTERIZATION OF *RHIZOCTONIA SOLANI* AND *RHIZOCTONIA*-LIKE FUNGI INFECTING VEGETABLES IN NEW YORK AND THEIR PATHOGENICITY TO CORN

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Mana Ohkura

May 2008

ABSTRACT

Vegetable growers in New York have recently observed that the corn rotation is no longer effective in suppressing diseases caused by *Rhizoctonia solani* and *Rhizoctonia*-like fungi. To investigate this problem, *R. solani* and *Rhizoctonia*-like fungi were isolated from naturally infected vegetables in New York. Sixty-eight isolates were genetically characterized and their pathogenicity to corn was determined under greenhouse conditions. Sequence analysis of the rDNA internal transcribed spacer region inferred 26 isolates to belong to *R. solani* anastomosis group (AG) 2-2 and 19 isolates to belong to AG 4. Remaining isolates belonged to AG 1, AG 2-1, AG 5, AG 11, *Ceratobasidium* AG (CAG) 2, CAG 6, and *Waitea circinata* var. *zeae*. This is a first report of AG 11 and *W. circinata* var. *zeae* recovered from naturally infected vegetables in New York. Pathogenicity trials on corn showed that the majority of isolates are pathogenic to corn and isolates belonging to AG 2-2 exhibited high virulence and isolates belonging to CAG 2 exhibited low virulence. These results suggest that certain strains of *R. solani* and *Rhizoctonia*-like fungi infecting vegetables in New York have acquired the ability to infect corn. In particular, isolates of AG 2-2 have been previously confirmed to produce the sexual stage under field conditions, suggesting that these isolates may have evolved to infect corn through sexual recombination. In addition, snap bean was inoculated with isolates exhibiting variable virulence on corn and a potential correlation between virulence on corn and snap bean was observed.

BIOGRAPHICAL SKETCH

Mana Ohkura was born on July 13, 1982 in Kasugai, Japan. As a child, she lived in the USA, India, and Japan. In 2001, she entered University of California, Davis and pursued a major in Biological Sciences with a Fungal Biology and Ecology minor. Her enjoyable experience working in Dr. Tom Gordon's lab and going mushroom hunting with Dr. Mike Davis got her very interested in Mycology and Plant Pathology. In 2005, Mana came to Cornell University and joined Dr. George Abawi's program in the Department of Plant Pathology. At Cornell, she worked on *Rhizoctonia solani* and *Rhizoctonia*-like fungi that infects vegetables in New York.

to Dad

ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. George Abawi, Dr. Kathie Hodge, and Dr. Chris Smart, for their time and input during my time at Cornell University. George has taught me about plant diseases and issues that growers deal with in the field. I appreciate his suggestions and patience when I was not sure about where to go with my project. Kathie has taught me a lot of interesting facts about fungi and I very much appreciate her support when I was confused about my career. I would also like to thank her for mentoring me during my teaching assistantships which made me realize the fun of teaching. Chris has guided me through all of the molecular work. I greatly appreciated her advice when I was having problems with trouble-shooting and her suggestions have significantly helped me complete my thesis. In addition to my committee members, I would like to thank members of the Abawi lab, John Ludwig and Beth Gugino, for their support. The Smart lab, Ning Zhang, Maryann Herman, Tanya Taylor, and Holly Lange, provided valuable technical assistance in the molecular characterization of isolates. I am also grateful to Dr. John Barnard for providing expert assistance in statistics.

TABLE OF CONTENTS

	Page
Biographical Sketch	iii
Dedication	iv
Acknowledgments	v
List of Tables	vi
List of Figures	vii
Chapter 1. Introduction	1
Systematics of *Rhizoctonia*	1
Biology and Control of *Rhizoctonia solani* and *Rhizoctonia*-like fungi	6
Rhizoctonia solani and *Rhizoctonia*-like Fungi Infecting Vegetables in New York	9
Chapter 2. Characterization of *Rhizoctonia solani* and *Rhizoctonia*-like Fungi Infecting Vegetables in New York and their Pathogenicity to Corn	21
Introduction	21
Materials and Methods	24
Isolate Collection	24
Molecular Characterization	27
Phylogenetic Analysis	30
Pathogenicity Evaluation on Corn in the Greenhouse	35
Pathogenicity Evaluation on Snap Bean in the Greenhouse	39
Statistical Analysis	39
Results	42
Phylogenetic Analysis	42
Pathogenicity Evaluation on Corn in the Greenhouse	45
Pathogenicity Evaluation on Snap Bean in the Greenhouse	51
Discussion	51
References	57

LIST OF TABLES

		Page
Table 1.1	Genera with *Rhizoctonia*-like anamorphs, and their phylogenetic affiliation.	2
Table 1.2	Anastomosis group, subgroup, and cultural type of *Rhizoctonia solani* and their common hosts.	7
Table 2.1	*Rhizoctonia solani* and *Rhizoctonia*-like fungi of New York characterized in this study.	25
Table 2.2	Polymerase chain reaction primer combinations, cloning status, sequencing primer(s), and GenBank accession numbers for individual isolates.	28
Table 2.3	Reference sequences used to characterize *Rhizoctonia solani* and *Rhizoctonia*-like fungi isolated in New York.	31
Table 2.4	Results from corn and snap bean pathogenicity trials and inferred identification of individual isolates.	48

LIST OF FIGURES

		Page
Figure 2.1	Inoculation of corn seedlings.	36
Figure 2.2	Incubation of corn seedlings.	37
Figure 2.3	Disease rating scale used in the corn pathogenicity trial.	38
Figure 2.4	Disease rating scale used in the snap bean pathogenicity trial.	40
Figure 2.5	Morpological diversity of *Rhizoctonia solani* and *Rhizoctonia*-like fungi collected in New York.	41
Figure 2.6	Neighbor-joining tree of reference sequences and collected New York isolates.	43
Figure 2.7	Bayesian inference tree of reference sequences and collected New York isolates.	46
Figure 2.8	Disease severity on corn caused by isolates collected in New York, when grouped by inferred anastomosis group, subgroup, or species.	52

CHAPTER 1

Introduction

Species of the form genus *Rhizoctonia* are diverse and ubiquitous in the soil, often associated with plant roots. Some are mycorrhizal, most are saprobic, and many are economically important plant pathogens that occur globally and cause disease on a wide range of hosts (Garcia et al. 2006; Sneh et al. 1996). However, studying these fungi has been a challenge due to their ambiguous taxonomy (Cubeta and Vilgalys 1997).

Systematics of Rhizoctonia

The form genus *Rhizoctonia* traditionally includes filamentous soil fungi that do not produce asexual spores, possess brown pigmented hyphae, and possess right-angled branching points with constrictions. The use of such general vegetative features as taxonomic characters has resulted in a taxon that includes a heterogeneous mix of polyphyletic fungi (Garcia et al. 2006; Stalpers and Andersen 1996). These fungi produce a diversity of teleomorphs, but the difficulty in inducing the teleomorphs limited *Rhizoctonia* taxonomy to be primarily dependent on anamorph features (Garcia et al. 2006). Scientists have studied morphological and ultrastructural features, hyphal anastomosis reactions, and nuclear condition to better understand *Rhizoctonia* systematics (Andersen 1996; Moore 1987, 1996; Parmeter et al. 1967; Tu and Kimbrough 1978; Tu et al. 1977). The discovery of various teleomorphs has cleared up some of the taxonomic ambiguity and currently, members of the form genus *Rhizoctonia sensu lato* have been segregated into at least seven teleomorphic genera (Garcia et al. 2006; Tu and Kimbrough 1978). The teleomorphs within the form genus *Rhizoctonia* and their corresponding anamorphs are listed in Table 1.1. Most fungi in the form genus *Rhizoctonia* belong to the basidiomycetes;

Table 1.1
Genera with *Rhizoctonia*-like anamorphs, and their phylogenetic affiliation.

Phylum	Teleomorph	Anamorph
Ascomycota	*Tricharina* Eckblad	*Ascorhizoctonia* Yang & Korf
Basidiomycota	*Ceratobasidium* Rogers	*Ceratorhiza* Moore
	Waitea Warcup & P. H. B. Talbot	*Chrysorhiza* T. F. Andersen & Stalpers
	Tulasnella Schroeter	*Epulorhiza* Moore
	Sebacina Tulasne	*Opadorhiza* Moore
	Thanatephorus Donk	*Rhizoctonia* J. G. Kuhn
	Helicobasidium Pat	*Thanatophytum* Nees

however, some are ascomycetes (Moore 1987; Tu and Kimbrough 1978; Yang and Korf 1985). The genus *Thanatephorus* (Donk 1956) was erected for the teleomorph of *R. solani* (= *T. cucumeris*), the most widely studied species in the form genus *Rhizoctonia* (Garcia et al. 2006; Stalpers and Andersen 1996). The anamorphic genus *Moniliopsis* Ruhland was previously proposed for *Rhizoctonia*-like species with *Thanatephorus* and *Waitea* teleomorphs (Moore 1987). However, since the name *Rhizoctonia solani* is well-established in the literature of plant pathology, a formal proposal to conserve *R. solani* as the type species for *Rhizoctonia* was made (Stalpers et al. 1998) and was approved at the 2005 International Botanical Congress (Vienna Code) (McNeill et al. 2006). Therefore, currently *Rhizoctonia sensu stricto* refers to anamorphs with *Thanatephorus* teleomorphs, including *R. solani*. The term "binucleate *Rhizoctonia*" is often used to describe *Ceratobasidium* species, which have *Rhizoctonia*-like anamorphs containing two nuclei in each cell. In following sections of this thesis, fungi within the form genus *Rhizoctonia s. l.* will be referred to as "*R. solani* and *Rhizoctonia*-like fungi" as the thesis will deal with specific groupings within *R. solani* and fungi outside of *Rhizoctonia s. s*.

The most studied *Rhizoctonia* species, *R. solani*, is considered a species complex, comprised of many genetically distinct lineages. Hyphal fusion reactions have been used to recognize anastomosis groups (AGs) within the species complex (Anderson 1982). Isolates belong to the same AG if their hyphae grow toward each other and fuse; fused and adjacent cells may or may not remain alive. On the other hand, isolates belong to different AGs if their hyphae do not undergo fusion (Carling 1996; Vilgalys and Cubeta 1994). Currently, there are 14 AGs: AG 1 through AG 13 and AG-BI (Carling et al. 2002c). AG-BI consists of bridging isolates that each anastomose with more than one AG (Carling 1996). Presently, AGs 1, 2, 3, 4, 6, 8, 9, 11, 12 are further divided into subgroups based on additional criteria such as colony

morphology, genetic variability, biochemical properties, and pathogenicity (Carling 1996). AG subgroups are determined by different criteria depending on the particular AG. For example, subgroups within AG 1 (AG 1-IA to AG 1-ID) are based on colony morphology and pathogenicity, AG 2 subgroups (AG 2-1 to AG 2-4) are based on hyphal fusion frequency, AG 4 subgroups (AG 4-HGI to AG 4-HGIII) are based on DNA-DNA complementarity and fatty acid analysis, and AG 8 subgroups (AG 8-ZG1 to AG 8-ZG5) are based on zymogram patterns (Carling 1996; Carling et al. 2002b; Johnk and Jones 2001; Naito and Kanematsu 1994; Priyatmojo et al. 2001). In addition, AG 2-2 is further subdivided into cultural types (AG 2-2IIIB, AG 2-2IV, AG 2-2 LP) according to pathogenicity and cultural morphology (Hyakumachi et al. 1998; Ogoshi 1987). AG 2-2IIIB was identified on mat rush (*Lomandra longifolia*) and referred to as the rush type, AG 2-2IV was identified on sugar beet and referred to as the root rot type, and AG 2-2LP was identified on warm season turf grasses and referred to as the large patch type (Hyakumachi et al. 1998; Ogoshi 1987). Similar to *R. solani*, *Ceratobasidium* and *Waitea* species have been subdivided into anastomosis groups (CAG/AG and WAG respectively) as well (Ogoshi et al. 1983b; Oniki et al. 1985). In the United States, *Ceratobasidium* species are divided into CAG 1 to CAG 7, while in Japan, they are divided into AG A to AG U (Burpee et al. 1980a, 1980b; Hyakumachi et al. 2005; Ogoshi et al. 1983a; Ogoshi et al. 1983b).

Characterizing *R. solani* and *Rhizoctonia*-like fungi by anastomosis reactions is time-consuming and can be ambiguous due to the presence of bridging isolates and isolates that have lost the ability to anastomose (Hyakumachi and Ui 1987; Sharon et al. 2006). Nutritional conditions can also affect anastomosis reactions (Yokoyama and Ogoshi 1988). Fortunately, the advance in molecular techniques has facilitated a more accurate classification of these fungi (Sharon et al. 2006). Various molecular markers have been developed to study different taxonomic levels of *R. solani* and *Rhizoctonia*-

like fungi (Cubeta and Vilgalys 1997; Johanson et al. 1998). In order to identify *R. solani* and *Rhizoctonia*-like fungi to AGs, currently DNA sequence analysis of the ribosomal RNA genes and in particular the internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal DNA (rDNA) are considered to be appropriate (Gonzalez et al. 2001; Sharon et al. 2006).

Studies have shown that it is possible to infer the species, AG, or subgroup of an unknown *R. solani* or *Rhizoctonia*-like fungus by constructing phylogenetic trees with rDNA ITS sequences of previously characterized isolates (Kuramae et al. 2003; Kuramae et al. 2007; Lehtonen et al. 2008; Manici and Bonora 2007; Rinehart et al. 2007). These studies have been conducted using isolates recovered from various crops as well as diverse locations suggesting the wide applicability of this protocol. The rDNA ITS sequence has also been used to study the phylogenetic relationships of already established AG, subgroups, and cultural types (Carling et al. 2002b; Gonzalez et al. 2006; Gonzalez et al. 2001; Kuninaga et al. 1997; Pope and Carter 2001; Salazar et al. 2000; Salazar et al. 1999; Sharon et al. 2006; Toda et al. 2004; Vilgalys and Cubeta 1994). Such studies have revealed contradictions between molecular and traditional systematics of *R. solani* and *Rhizoctonia*-like fungi: Two of these studies showed that subgroups within AG 2 are phylogenetically distant from each other, suggesting that anastomosis reactions are not good indicators of evolutionarily distinct units (Kuninaga et al. 1997; Vilgalys and Cubeta 1994). Another study showed that *Ceratobasidium* AGs may be polyphyletic in origin and that certain CAGs are more closely related to *R. solani* AGs (Gonzalez et al. 2001). The rDNA ITS sequence has also been used to characterize and propose new AGs and subgroups (Carling et al. 2002b; Carling et al. 2002c; Kuninaga et al. 2000). Thus, the rDNA ITS region appears to be useful in demonstrating phylogenetic relationships of *R. solani* and *Rhizoctonia*-like fungi.

Biology and Control of Rhizoctonia solani and Rhizoctonia-like fungi

Rhizoctonia solani and *Rhizoctonia*-like fungi are geographically distributed worldwide and include some of the world's most devastating plant pathogens. Different AGs and species have different host ranges and most vascular plants are potential hosts of plant pathogenic *R. solani* or *Rhizoctonia*-like fungi (Ogoshi 1996; Roberts 1999). Common symptoms caused by these fungi include damping off, root rot, stem rot, foliar blight, and stem canker (Agrios 2005). The widely studied species *R. solani* has a very wide host range and can infect vegetables, grasses, ornamentals, fruit trees as well as pine trees (Garcia et al. 2006; Gonzalez et al. 2006). Some AGs have a wide host range, while other AGs have a narrow host range (Ogoshi 1996). Table 1.2 summarizes the different AGs, subgroups, and cultural types and their corresponding common hosts (Garcia et al. 2006; Sneh et al. 1991). Many *R. solani* and *Rhizoctonia*-like fungi produce sclerotia that come in various shapes and sizes (Roberts 1999; Sumner 1996). Sclerotia are resistant, asexual propagules that allow survival in the soil for several years and can also serve as a source of inoculum (Roberts 1999; Sherwood 1970; Sumner 1996). In *Thanatephorus* species, "loose type" sclerotia are formed that lack a distinct rind (Tu and Kimbrough 1975). Under certain field conditions such as high relative humidity, *T. cucumeris* produces sexual propagules, known as basidiospores, that cause aerial infections. In contrast to asexual propagation by mycelia and sclerotia, disease spread is considered to be faster by basidiospores (Naito 1996). Furthermore, sexual reproduction can increase gene diversity through recombination and therefore is more likely to yield new combinations of virulence genes to overcome plant resistance (Agrios 2005). On the other hand, some *R. solani* and *Rhizoctonia*-like fungi are hypovirulent or non-pathogenic. These strains are studied for their potential use in biocontrol. Most of

Table 1.2
Anastomosis group (AG), subgroup, and cultural type of *Rhizoctonia solani* and their common hosts (Garcia et al. 2006; Mazzola et al. 1996; Sneh et al. 1991; Tu et al. 1996).

AG / Subgroup	Hosts
AG 1 IA	rice, corn sorghum, bean, soybean, turfgrass, camphor seedlings, crimson clover
AG 1 IB	bean, rice, soybean, leguminous woody plants, lettuce, hortensia, cabbage, figs
AG 1 IC	buckwheat, carrot, soybean, flax, pine, lettuce
AG 2-1	Crucifers, strawberry, tulip, Japanese radish, subterranean clover
AG 2-2IIIB	rice, mat rush, ginger, turfgrass, corn, sugar beet, Chrysanthemum, Gladiolus, edible burdock, tree seedlings, soybean
AG 2-2IV	sugar beet, turfgrass
AG 2-3	Soybean
AG 3 (PT, TB)	potato, tabacco, tomato, egg plant, pepper
AG 4 (HGI, HGII, HGIII)	tomato, pea, potato, soybean, onion, cotton,snap bean, Loblolly pine seedlings, peanut, slash pine, cucumber, corn
AG 5	potato, turfgrass, bean, soybeans,
AG 6 (HG-I, GV)	non pathogenic
AG 7	Soybeans
AG 8 (ZG 1-1, ZG 1-2, ZG 1-3,ZG 1-4, ZG 1-5)	*Poaceae*, cereals
AG 9 (TP, TX)	crucifers, potato
AG 10	non pathogenic
AG 11	Wheat
AG 12	cauliflower, radish
AG 13	non pathogenic
AG BI	non pathogenic

these are *Ceratobasidium* species, but certain *R. solani* AGs are avirulent as well, such as AG 10 (Sneh 1998).

Control of plant diseases caused by *R. solani* and *Rhizoctonia*-like fungi is difficult due to various biological properties of the pathogen. Their wide host ranges and versatility has made breeding for resistant cultivars difficult and their capability to adapt allows great potential for the pathogen to overcome ecological changes (Baker 1970; Leach and Garber 1970; Ogoshi 1996). The most popular control method is chemical control and growers highly depend on fungicides to suppress diseases caused by *R. solani* and *Rhizoctonia*-like fungi (Kataria and Gisi 1996). However, disease control using fungicides is not always effective. Satisfactory disease control can be hard to achieve due to the pathogens' soil-borne nature, difficulty in timing of fungicide application, and taxonomic complexity among *R. solani* and *Rhizoctonia*-like fungi (Katan 1996; Kataria and Gisi 1996; Olaya et al. 1994). In addition, even if a fungicide is known to be effective, growers will prefer not to use it if it is expensive (Abawi, personal communication). Therefore, crop rotation is commonly recommended as a method to control diseases caused by *R. solani* and *Rhizoctonia*-like fungi (Huber and Sumner 1996). For vegetables, grain crop rotations are suggested to suppress diseases caused by *R. solani* (Leach and Garber 1970; Reiners and Petzoldt 2006).

Rhizoctonia solani and Rhizoctonia-like fungi Infecting Vegetables in New York

Rhizoctonia solani and *Rhizoctonia*-like fungi cause root and foliar diseases on various vegetables such as beans, table beets, carrots, and cabbage in New York State. The importance of the pathogen in New York has increased since the discovery of its perfect state, *T. cucumeris* on table beets in 1990 (Olaya and Abawi 1991). Subsequently, *T. cucumeris* was identified on snap beans and other crops in the state

as well (Abawi et al. 1995). The damage caused by *R. solani* and related fungi in New York has increased steadily during the past 10 years and the production of large numbers of aerially dispersed basidiospores is thought to be one of the contributing factors (Olaya and Abawi 1994b). Another factor is changes in cultural practices where large tractors throw infested soil onto crowns of the plants as they cultivate the field for weed control (Olaya and Abawi 1994b).

Until recently, grain crop rotations were effective in suppressing vegetable diseases caused by *R. solani* and *Rhizoctonia*-like fungi (Abawi and Ludwig 2005; Reiners and Petzoldt 2006). However, during the past few years New York growers have reported that the corn rotation has been ineffective (Abawi, personal communication). A similar problem has been reported in Germany, where disease caused by *R. solani* on sugar beets has increased in locations where narrow rotations of sugar beet and corn are practiced. They demonstrated that the main strain known to be problematic on sugar beet in Europe, AG-2-2IIIB, has the ability to infect sugar beet after surviving on corn residues in the field (Ithurrart et al. 2004). Additionally, Win and Sumner (1988) have shown that disease on beans is more severe when beans are planted after corn that was infested with AG-4 and AG-2-2 (Win and Sumner 1988).

Among *R. solani* and *Rhizoctonia*-like fungi, isolates belonging to AG 1, AG 2, AG 4, and *W. circinata* var. *zeae* are known to cause disease on corn (Garcia et al. 2006; Mazzola et al. 1996; Sneh et al. 1991; Sumner and Bell 1982a, 1982b). Within AG 1 and AG 2, subgroups AG 1-IA and AG 2-2 have been well documented to infect corn (Garcia et al. 2006; Li et al. 1998; Sneh et al. 1991; Sumner and Bell 1982b). Furthermore, cultural type AG 2-2IIIB has been reported to be the causal agent of root rot on corn in the United States (Ithurrart et al. 2004). Previous studies in New York have shown that the dominant AGs present in western New York are AG 2-2 on table

beet (88%) (Olaya and Abawi 1994a), AG-4 on snap bean (55%) (Galindo et al. 1982), and AG 1 on cabbage (Abawi and Martin 1985). Other isolates found associated with table beets belonged to AG-5, AG-4, AG-2-1, and binucleate *Rhizoctonia* (Olaya and Abawi 1994a), and those associated with snap beans belonged to AG-1 and AG-2 (Galindo et al. 1982). One possible explanation why the corn rotation is ineffective may be due to the existence of undetected *R. solani* subgroups or cultural types that are pathogenic on corn. Previous characterization in this region showed that AG and subgroups pathogenic on corn, AG 1, AG 2-2, and AG 4 are present. However, these studies were conducted more than 10 years ago and isolates were not identified to further subgroup or cultural type. If AG 1-IA and AG 2-2IIIB can be proven to be present, it would confirm the presence of well-documented strains pathogenic to corn. As for AG 4, specific subgroups known to be pathogenic to corn are not well established.

A second possible reason for the ineffectiveness of the corn rotation may be that isolates that cause disease on vegetables have adapted to survive on corn. There have been two reports of AG-5, a group not commonly found infecting corn, causing infection on corn (Li et al. 1998; Tomaso-Peterson and Trevathan 2007). Conversely, isolates that usually infect corn may have acquired the ability to infect vegetables. For example, isolates of the *Rhizoctonia*-like fungus, *W. circinata* var. *zeae* are known to be pathogenic to corn (Mazzola et al. 1996; Sneh et al. 1991). However, *W. circinata* var. *zeae* has been found to cause disease on onions (Erper et al. 2006) and has been isolated from soybean and bean in Turkey (Erper et al. 2005). Lastly, a non-pathogenic AG or species may have emerged with the ability to infect these crops. For instance, AG-13, usually reported to be non-pathogenic, has been isolated from corn in Mississippi (Garcia et al. 2006; Tomaso-Peterson and Trevathan 2004b). Accurate assessment of present subdivisions of *R. solani* and *Rhizoctonia*-like fungi in

New York and their effect on corn may clarify some of the questions raised above. The aim of this study was to characterize the *R. solani* and *Rhizoctonia*-like fungi present in New York vegetable field soils using phylogenetic analysis of the rDNA ITS sequences, and to evaluate corn as a potential host. Results from these experiments can be used to test the following hypotheses to explain the ineffectiveness of the corn rotation:

H1. Isolates of *R. solani* known to be pathogenic to corn, AG 1-IA and AG 2-2IIIB, exist in this region.

H2. Isolates of *R. solani* and *Rhizoctonia*-like fungi that infect vegetables have gained the ability to infect corn.

H3. Non-pathogenic isolates of *R. solani* and *Rhizoctonia*-like fungi have developed the ability to infect vegetables and corn.

REFERENCES

Abawi, G. S., and S. B. Martin. 1985. *Rhizoctonia* foliar blight of cabbage in New York State. *Plant Disease* 69 (2):158-161.

Abawi, G. S., and J. W. Ludwig. 2005. Effect of three crop rotations with and without deep plowing on root rot severity and yield of beans. *Annual Report of the Bean Improvement Cooperative* 48:118-119.

Abawi, G. S., G. Olaya, and J. W. Ludwig. 1995. Occurrence of *Thanatephorus cucumeris* on snap beans in New York. *Phytopathology* 85 (12):1554.

Agrios, G. N. 2005. *Plant pathology* Fifth edition ed. New York: Elsevier Academic Press.

Andersen, T. F. 1996. A comparative taxonomic study of *Rhizoctonia* sensu lato employing morphological, ultrastructural and molecular methods. *Mycological Research* 100:1117-1128.

Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology* 20:329-347.

Baker, K. F. 1970. Types of *Rhizoctonia solani* diseases and their occurrence. In *Rhizoctonia solani: Biology and Pathology*, edited by J. R. J. Parmeter. Berkeley: University of California Press.

Burpee, L. L., P. L. Sanders, H. Jr Cole, and R. T. Sherwood. 1980a. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. *Mycologia* 72 (4):689-701.

Burpee, L. L., P. L. Sanders, H. Jr Cole, and R. T. Sherwood. 1980b. Pathogenicity of *Ceratobasidium cornigerum* and related fungi representing 5 anastomosis groups. *Phytopathology* 70 (9):843-846.

Carling, D. E. 1996. Grouping of *Rhizoctonia solani* by hyphal anastomosis reaction. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control*, edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Carling, D. E., S. Kuninaga, and K. A. Brainard. 2002a. Hyphal Anastomosis Reactions, rDNA-Internal Transcribed Spacer Sequences, and Virulence Levels Among Subsets of Rhizoctonia solani Anastomosis Group-2 (AG-2) and AG-BI.

Carling, D. E., R. E. Baird, R. D. Gitaitis, K. A. Brainard, and S. Kuninaga. 2002b. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology* 92 (8):893-899.

Cubeta, M. A., and R. Vilgalys. 1997. Population biology of the *Rhizoctonia solani* complex. *Phytopathology* 87 (4):480-484.

Donk, M. A. 1956. Notes on resupinate Hymenomycetes - II. The tulasnelloid fungi. *Reinwardtia* 3 ((3)):363-379.

Erper, I., G. Karaca, and I. Ozkoc. 2005. First report of root rot of bean and soybean caused by *Rhizoctonia zeae* in Turkey. *Plant Disease* 89 (2):203.

Erper, I., G. H. Karaca, M. Turkkan, and I. Ozkoc. 2006. Characterization and pathogenicity of *Rhizoctonia* spp. from onion in Amasya, Turkey. *Journal of Phytopathology* 154 (2):75-79.

Galindo, J. J., G. S. Abawi, and H. D. Thurston. 1982. Variability among isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York. *Plant Disease* 66 (5):390-394.

Garcia, V. G., M. A. P. Onco, and V. R. Susan. 2006. Review. Biology and systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research* 4 (1):55-79.

Gonzalez, D., M. A. Cubeta, and R. Vilgalys. 2006. Phylogenetic utility of indels within ribosomal DNA and beta-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. *Molecular Phylogenetics and Evolution* 40 (2):459-470.

Gonzalez, D., D. E. Carling, S. Kuninaga, R. Vilgalys, and M. A. Cubeta. 2001. Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia* 93 (6):1138-1150.

Huber, Don M., and Donald R. Sumner. 1996. Suppressive soil amendments for the control of *Rhizoctonia* species. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control*, edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Hyakumachi, M., and T. Ui. 1987. Non-self-anastomosing isolates of *Rhizoctonia solani* obtained from fields of sugar-beet monoculture. *Transactions of the British Mycological Society* 89:155-159.

Hyakumachi, M., A. Priyatmojo, M. Kubota, and H. Fukui. 2005. New anastomosis groups, AG-T and AG-U, of binucleate Rhizoctonia spp. causing root and stem rot of cut-flower and miniature roses. *Phytopathology* 95 (7):784-792.

Hyakumachi, M., T. Mushika, Y. Ogiso, T. Toda, K. Kageyama, and T. Tsuge. 1998. Characterization of a new cultural type (LP) of *Rhizoctonia solani* AG2-2 isolated from warm-season turfgrasses, and its genetic differentiation from other cultural types. *Plant Pathology (Oxford)* 47 (1):1-9.

Ithurrart, M. E. F., G. Buttner, and J. Petersen. 2004. *Rhizoctonia* root rot in sugar beet (*Beta vulgaris* ssp *altissima*) - Epidemiological aspects in relation to maize (*Zea mays*) as a host plant. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 111 (3):302-312.

Johanson, Andrea, Helen C. Turner, Gareth J. McKay, and Averil E. Brown. 1998. A PCR-based method to distinguish fungi of the rice sheath-blight complex, *Rhizoctonia solani*, *R. oryzae* and *R. oryzae-sativae*. *FEMS Microbiology Letters* 162 (2):289-294.

Johnk, Janell Stevens, and Roger K. Jones. 2001. Differentiation of three homogeneous groups of *Rhizoctonia solani* anastomosis group 4 by analysis of fatty acids. *Phytopathology* 91 (9):821-830.

Katan, Jaacov. 1996. Soil solarization for the control of diseases caused by *Rhizoctonia* spp. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control*, edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Kataria, H. R., and U. Gisi. 1996. Chemical control of *Rhizoctonia* species. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Dordrecht / Boston / London: Kluwer Academic Publisher.

Kuninaga, S., T. Natsuaki, T. Takeuchi, and R. Yokosawa. 1997. Sequence variation of the rDNA ITS regions within and between anastomosis groups in *Rhizoctonia solani*. *Current Genetics* 32 (3):237-243.

Kuninaga, Shiro, Donald E. Carling, Toru Takeuchi, and Ryozo Yokosawa. 2000. Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG-3. *Journal of General Plant Pathology* 66 (1):2-11.

Kuramae, E. E., A. L. Buzeto, M. B. Ciampi, and N. L. Souza. 2003. Identification of *Rhizoctonia solani* AG 1-IB in lettuce, AG 4 HG-I in tomato and melon, and AG 4 HG-III in broccoli and spinach, in Brazil. *European Journal of Plant Pathology* 109 (4):391-395.

Kuramae, E. E., A. L. Buzeto, A. K. Nakatani, and N. L. Souza. 2007. rDNA-based characterization of a new binucleate *Rhizoctonia* spp. causing root rot on kale in Brazil. *European Journal of Plant Pathology* 119 (4):469-475.

Leach, L. D., and R. H. Garber. 1970. Control of *Rhizoctonia solani*. In *Rhizoctonia solani: Biology and Pathology*, edited by J. R. J. Parmeter. Berkeley: University of California Press.

Lehtonen, M. J., P. Ahvenniemi, P. S. Wilson, M. German-Kinnari, and J. P. T. Valkonen. 2008. Biological diversity of *Rhizoctonia solani* (AG-3) in a northern potato-cultivation environment in Finland. *Plant Pathology* 57 (1):141-151.

Li, Wu, and Yan. 1998. Aetiology of *Rhizoctonia* in sheath blight of maize in Sichuan. *Plant Pathology* 47 (1):16-21.

Manici, L. M., and P. Bonora. 2007. Molecular genetic variability of Italian binucleate *Rhizoctonia* spp. isolates from strawberry. *European Journal of Plant Pathology* 118 (1):31-42.

Mazzola, M., R. W. Smiley, A. D. Rovira, and R. J. Cook. 1996. Chracterization of *Rhizoctonia* isolates, disease occurence and management in cereals. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Dordrecht / Boston / London: Kluwer Academic Publisher.

McNeill, J., F. R. Barrie, H. M. Burdet, V. Demoulin, D. L. Hawksworth, K. Marhold, D. H. Nicolson, J. Prado, P. C. Silva, J. E. Skog, J. H. Wiersema, and N. J. Turland. 2006. International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress. In *International Botanical Congress*. Vienna, Austria: International Association for Plant Taxonomy

Moore, Royall T. 1987. The Genera of *Rhizoctonia*-like fungi: *Ascorhizoctonia*, *Ceratorhiza gen. nov.*, *Epulorhiza gen. nov.*, *Moniliopsis,* and *Rhizoctonia*. *Mycotaxon* 29:91-99.

Moore, Royall T. 1996. The dolipore/parenthesome septum in modern taxonomy. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Naito, S. 1996. Basidiospore dispersal and survival. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited

by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Naito, Shigeo, and Seiji Kanematsu. 1994. Characterization and pathogenicity of a new anastomosis subgroup AG-2-3 of *Rhizoctonia solani* Kuhn isolated from leaves of soybean. *Annals of the Phytopathological Society of Japan* 60 (6):681-690.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. In *Annual Review of Phytopathology, Vol. 25*, edited by R. J. Cook. Palo Alto, California: Annual Reviews Inc.

Ogoshi, A. 1996. Introduction - The genus *Rhizoctonia*. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Ogoshi, A., M. Oniki, T. Araki, and T. Ui. 1983a. Anastomosis groups of binucleate *Rhizoctonia* in Japan and North America and their perfect states. *Nippon Kingakukai Kaiho* 24 (1):79-88.

Ogoshi, A., Oniki M., Araki T., and Ui T. 1983b. Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect states. *Journal of the Faculty of Agriculture Hokkaido University* 61 (2):244-260.

Olaya, G., and G. S. Abawi. 1991. Occurrence of *Thanatephorus cucumeris* on table beets in New York state. *Phytopathology* 81 (10):1186.

Olaya, G., and G. S. Abawi. 1994a. Characteristics of *Rhizoctonia solani* and binucleate *Rhizoctonia* species causing foliar blight and root rot on table beets in New York state. *Plant Disease* 78 (8):800-804.

Olaya, G., and G. S. Abawi. 1994b. Influence of inoculum type and moisture on development of *Rhizoctonia solani* on foliage of table beets. *Plant Disease* 78 (8):805-810.

Olaya, G., G. S. Abawi, and J. Barnard. 1994. Response of *Rhizoctonia solani* and binucleate *Rhizoctonia* to 5 fungicides and control of pocket rot of table beets with foliar sprays. *Plant Disease* 78 (11):1033-1037.

Oniki, M., A. Ogoshi, T. Araki, R. Sakai, and S. Tanaka. 1985. The perfect state of *Rhizoctonia oryzae* and *Rhizoctonia zeae*, and the anastomosis groups of *Waitea circinata*. *Transactions of the Mycological Society of Japan* 26 (2):189-198.

Parmeter, J. R., H. S. Whitney, and W. D. Platt. 1967. Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. *Phytopathology* 57 (2):218-223.

Pope, E. J., and D. A. Carter. 2001. Phylogenetic placement and host specificity of mycorrhizal isolates belonging to AG-6 and AG-12 in the *Rhizoctonia solani* species complex. *Mycologia* 93 (4):712-719.

Priyatmojo, Achmadi, Verma E. Escopalao, Naomi G. Tangonan, Cecilia B. Pascual, Haruhisa Suga, Koji Kageyama, and Mitsuro Hyakumachi. 2001. Characterization of a new subgroup of *Rhizoctonia solani* anastomosis group 1 (AG-1-ID), causal agent of a necrotic leaf spot on coffee. *Phytopathology* 91 (11):1054-1061.

Reiners, S., and C. H. Petzoldt, eds. 2006. *2006 Integrated crop and pest management guidelines for commercial vegetable production*. Ithaca: Cornell University

Rinehart, T. A., W. E. Copes, T. Toda, and M. A. Cubeta. 2007. Genetic characterization of binucleate *Rhizoctonia* species causing web blight on *Azalea* in Mississippi and Alabama. *Plant Disease* 91 (5):616-623.

Roberts, Peter. 1999. *Rhizoctonia-forming fungi*. Kew: Royal Botanic Gardens.

Salazar, Oscar, Maria C. Julian, Mitsuro Hyakumachi, and Victor Rubio. 2000. Phylogenetic grouping of cultural types of *Rhizoctonia solani* AG 2-2 based on ribosomal ITS sequences. *Mycologia* 92 (3):505-509.

Salazar, Oscar, Johannes H. M. Schneider, Maria C. Julian, Jaap Keijer, and Victor Rubio. 1999. Phylogenetic subgrouping of *Rhizoctonia solani* AG 2 isolates based on ribosomal ITS sequences. *Mycologia* 91 (3):459-467.

Sharon, Michal, Shiro Kuninaga, Mitsuro Hyakumachi, and Baruch Sneh. 2006. The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. *Mycoscience* 47 (6):299-316.

Sherwood, R. T. 1970. Physiology of *Rhizoctonia solani*. In *Rhizoctonia solani: Biology and Pathology*, edited by J. R. J. Parmeter. Berkeley: University of California Press

Sneh, Baruch. 1998. Use of non-pathogenic or hypovirulent fungal strains to protect plants against closely related fungal pathogens. *Biotechnology Advances* 16 (1):1-32.

Sneh, Baruch, L. Burpee, and A. Ogoshi. 1991. *Identification of Rhizoctonia species*. St. Paul: APS Press.

Sneh, Baruch, Suha Jajabi-Hare, Stephen Neate, and Gerda Dijst, eds. 1996. *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.* Boston: Kluwer Academic Publishers.

Stalpers, J. A., and T. F. Andersen. 1996. A synopsis of the taxonomy of teleomorphs connected with *Rhizoctonia* s.l. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Stalpers, J. A., T. F. Andersen, and W. Gams. 1998. Two proposals to conserve the names *Rhizoctonia* and *R. solani* (Hyphomycetes). *Taxon* 47 (3):725-726.

Sumner, D. R. 1996. Sclerotia formation by *Rhizoctonia* species and their survival. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Sumner, D. R., and D. K. Bell. 1982a. Crop rotation and yield loss in corn in soil infested with *Rhizoctonia solani* AG-2 and AG-4. *Phytopathology* 72 (3):361-362.

Sumner, D. R., and D. K. Bell. 1982b. Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. *Phytopathology* 72 (1):86-91.

Toda, T., J. M. Mghalu, A. Priyatmojo, and M. Hyakumachi. 2004. Comparison of sequences for the internal transcribed spacer region in *Rhizoctonia solani* AG 1-ID and other subgroups of AG 1. *Journal of General Plant Pathology* 70:270-272.

Tomaso-Peterson, M., and L. E. Trevathan. 2004. Rhizoctonia solani AG-13 Isolated from Corn in Mississippi.

Tomaso-Peterson, M., and L. E. Trevathan. 2007. Characterization of *Rhizoctonia*-like fungi isolated from agronomic crops and turfgrasses in Mississippi. *Plant Disease* 91 (3):260-265.

Tu, C. C., and J. W. Kimbrough. 1975. Morphology, development, and cytochemistry of hyphae and sclerotia of species in *Rhizoctonia* complex. *Canadian Journal of Botany-Revue Canadienne De Botanique* 53 (20):2282-2296.

Tu, C. C., and J. W. Kimbrough. 1978. Systematics and phylogeny of fungi in the *Rhizoctonia* complex. *Botanical Gazette* 139 (4):454-466.

Tu, C. C., J. W. Kimbrough, and H. C. Aldrich. 1977. Cytology and ultrastructure of *Thanatephorus cucumeris* and related taxa of *Rhizoctonia* complex. *Canadian Journal of Botany-Revue Canadienne De Botanique* 55 (18):2419-2436.

Tu, Chin-Chyu, Ting-Fang Hsieh, and Yih-Chang Chang. 1996. Vegetable diseases incited by *Rhizoctonia* spp. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Vilgalys, R., and M. A. Cubeta. 1994. Molecular systematics and population biology of *Rhizoctonia*. *Annual Review of Phytopathology* 32:135-155.

Win, H. H., and D. R. Sumner. 1988. Root rot induced in snap bean by *Rhizoctonia solani* AG-4 and AG-2 type 2 in conservation tillage following corn. *Plant Disease* 72 (12):1049-1053.

Yang, C. S., and R. P. Korf. 1985. *Ascorhizoctonia* new-genus and *Complexipes* emend., two genera for anamorphs of species assigned to *Tricharina* discomycetes. *Mycotaxon* 23:457-482.

Yokoyama, K., and A. Ogoshi. 1988. Studies on hyphal anastomosis of *Rhizoctonia solani*. V. Nutritional conditions for anastomosis. *Nippon Kingakukai Kaiho* 29 (2):125-132.

CHAPTER 2

Characterization of *Rhizoctonia solani* and *Rhizoctonia*-like Fungi Infecting Vegetables in New York and their Pathogenicity to Corn

Introduction

Rhizoctonia solani and *Rhizoctonia*-like fungi cause root and foliar diseases on various vegetables such as beans, table beets, carrots and cabbage in New York State and other production regions. The damage caused by these fungi in New York on vegetables has increased steadily during the past 10 years. The sexual stage of *R. solani*, *Thanatephorus cucumeris,* was first reported in 1990 in New York on table beets (Olaya and Abawi 1991) and may be one of the contributing factors to increased damage by *Rhizoctonia*-incited diseases through large numbers of aerially dispersed basidiospores (Olaya and Abawi 1994b). Subsequently, *T. cucumeris* was observed on snap beans and other crops in the state (Abawi et al. 1995). Another factor for the increased prevalence of *Rhizoctonia* infections was thought to be changes in cultural practices, in which large tractors throw infested soil onto crowns of the plants as they cultivate fields for weed control (Olaya and Abawi 1994b).

Control of diseases caused by *R. solani* and *Rhizoctonia*-like fungi is difficult due to their soilborne nature, ability to persist as sclerotia, wide host range and versatility (Baker 1970; Katan 1996; Leach and Garber 1970; Menzies 1970; Ogoshi 1996). Today, many growers depend on fungicides to control *Rhizoctonia* diseases, but they are costly and must be applied at planting or early in the growing season to be effective (Olaya et al. 1994). For vegetable growers, grain crop rotations are recommended and have been effective in suppressing diseases caused by *R. solani* and *Rhizoctonia*-like fungi until recently (Abawi and Ludwig 2005; Reiners and Petzoldt 2006). However, during the past few years growers have reported that grain crop

rotations have been ineffective (Abawi, personal communication). Increasing disease caused by these fungi and the limited control options available to growers warrant an in depth investigation of this problem. A similar problem has been reported in Germany where high levels of infection on sugar beet has been observed when the crop is in narrow rotation with corn (Ithurrart et al. 2004)

Among the anastomosis groups (AGs) of *R. solani*, isolates belonging to AG 1, AG 2, and AG 4 have been documented to be pathogenic on corn (Garcia et al. 2006; Sneh et al. 1991; Sumner and Bell 1982a, 1982b). In particular, subgroups AG 1-IA and AG 2-2 have been reported to infect corn and cultural type AG 2-2IIIB has been reported to be the causal agent for root rot on corn in the United States (Buddemeyer et al. 2004; Garcia et al. 2006; Ithurrart et al. 2004; Sneh et al. 1991). Previous studies in this region showed that the dominant AGs present in western New York were AG 2-2 on table beets (88%) (Olaya and Abawi 1994a), AG-4 on snap beans (55%) (Galindo et al. 1982), and AG 1 on cabbage (Abawi and Martin 1985). Other isolates found associated with table beets belonged to AG-5, AG-4, AG-2-1, and binucleate *Rhizoctonia* (= *Ceratobasidium* spp.) (Olaya and Abawi 1994a), whereas those from snap beans belonged to AG-1 and AG-2 (Galindo et al. 1982).

One explanation of why the corn rotation is ineffective may be the existence of undetected subgroups/cultural types of *R. solani* that are pathogenic on corn (such as AG 1-IA and AG 2-2IIIB). Previous characterizations of New York isolates of *R. solani* recovered from vegetables were conducted more than 10 years ago and although AG 1 and AG 2-2 were detected, they were not identified to further subgroups or cultural types. A second potential reason for the ineffectiveness of the corn rotation may be that the isolates that cause disease on vegetables have adapted to infect and/or survive on corn. As an example, AG 5 is commonly found infecting vegetables and turf grasses (Garcia et al. 2006; Sneh et al. 1991), but two recent

studies have reported its ability to infect corn (Li et al. 1998; Tomaso-Peterson and Trevathan 2007). In contrast, isolates that usually infect corn may have acquired the ability to infect vegetable crops. For instance, isolates of the *Rhizoctonia*-like species, *Waitea circinata*, are generally known to be pathogenic on grasses including corn (Sneh et al. 1991). However, *W. circinata* var. *zeae* has been found to cause disease on onions (Erper et al. 2006) and has been isolated from naturally infected soybean and bean in Turkey (Erper et al. 2005). Lastly, an AG or species not known to cause disease on either vegetables or corn may have emerged with the ability to infect these crops. For example, AG-13, usually thought to be non-pathogenic (Garcia et al. 2006), has been isolated from corn in Mississippi (Tomaso-Peterson and Trevathan 2004a).

Taxonomic ambiguity among *R. solani* and *Rhizoctonia*-like fungi has made studying these fungi challenging (Cubeta and Vilgalys 1997). However, advances in molecular techniques have made characterization of species and AGs easier. Currently, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) is considered most appropriate in characterizing these fungi (Gonzalez et al. 2001; Sharon et al. 2006). DNA sequence data from the ITS region have been used to characterize unknown isolates of *R. solani* and *Rhizoctonia*-like fungi to AGs (Kuramae et al. 2007; Lehtonen et al. 2008; Manici and Bonora 2007; Rinehart et al. 2007). To investigate the reasons responsible for the ineffectiveness of corn rotation to suppress *Rhizoctonia*-incited diseases on vegetables in New York, accurate assessment of the *R. solani* and *Rhizoctonia*-like fungal population present in the region is necessary. This study tested three hypotheses that may explain the ineffectiveness of corn rotation in New York: 1. Isolates of *R. solani* subgroups/cultural type known to be pathogenic to corn, AG 1-IA and AG 2-2IIIB, exist in this region. 2. Isolates of *R. solani* and *Rhizoctonia*-like fungi that infect

vegetables have gained the ability to infect corn. 3. Non-pathogenic isolates of *R. solani* and *Rhizoctonia*-like fungi have emerged with the ability to infect vegetable and corn. These hypotheses were tested by characterizing the *R. solani* and *Rhizoctonia*-like fungi infecting vegetables in New York and evaluating corn as a potential host.

Methods and Materials

Isolate Collection

One hundred and fifteen isolates of *R. solani* and *Rhizoctonia*-like fungi were recovered from symptomatic vegetable tissues throughout New York State (Table 2.1). Host plants included pea, snap bean, dry bean, cabbage, carrot, and table beet. The fungi were isolated by placing small pieces of infected tissue on acidified water agar medium (pH 3.5). Prior to placement on media, infected tissue pieces were soaked in 10% bleach for 40 seconds to eliminate superficial contaminants and the lesion margins were cut off using a sterile scalpel. Each isolate was established by making a hyphal tip transfer from the margin of a colony exhibiting typical colony morphology and hyphal branching patterns of *Rhizoctonia* under a dissecting microscope. Isolates were stored in vials on Potato Dextrose Agar (PDA) covered with mineral oil at -4 ºC. Sixty-eight isolates were chosen to represent *Rhizoctonia* and *Rhizoctonia*-like fungi causing disease on vegetables in New York for molecular characterization and pathogenicity trials (Table 2.1).

Table 2.1
Rhizoctonia solani and *Rhizoctonia*-like fungi of New York characterized in this study. Isolate number, host plant, location, and date of isolation are listed. Footnotes indicate the rotation crop that the field the isolate was recovered from was planted to in 2005.

Isolate	Host Plant	Location[a]	Date of collection
R1	snap bean	Ontario	Fall 2005
R2	snap bean	Ontario	Fall 2005
R3	carrot	Yates	June 2006
R4	cabbage	Seneca	June 2006
R5	pea	Ontario	June 2006
R6	pea	Ontario	June 2006
R7	pea	Genesee	June 2006
R8	table beet	Genesee	August 2004
R9	carrot	Unknown (CNY)	June 2004
R10	snap bean	Livingston	June 2006
R11	snap bean	Livingston	June 2006
R12	snap bean	Genesee	June 2006
R13[b]	snap bean	Genesee	June 2006
R14[b]	snap bean	Genesee	June 2006
R15	carrot	Orleans	June 2006
R16	carrot	Orleans	June 2006
R17	carrot	Orleans	June 2006
R18	carrot	Orleans	June 2006
R20	snap bean	Orleans	June 2006
R21	snap bean	Orleans	June 2006
R22	snap bean	Orleans	June 2006
R25	snap bean	Orleans	June 2006
R27	table beet	Livingston	July 2006
R29	table beet	Livingston	July 2006
R31	table beet	Livingston	July 2006
R32	table beet	Livingston	July 2006
R33	table beet	Livingston	July 2006
R35	dry bean	Livingston	July 2006
R36	dry bean	Livingston	July 2006
R37	dry bean	Livingston	July 2006
R39	table beet	Livingston	July 2006
R41	table beet	Livingston	July 2006
R43	snap bean	Chemung	July 2006
R47	table beet	Genesee	July 2006

Isolate	Host Plant	Location[a]	Date of collection
R55	snap bean	Genesee	July 2006
R57	snap bean	Genesee	July 2006
R59	snap bean	Ontario	July 2006
R60	snap bean	Ontario	July 2006
R62	table beet	Ontario	July 2006
R64	snap bean	Genesee	July 2006
R65	snap bean	Genesee	July 2006
R66[c]	table beet	Genesee	July 2006
R68[c]	table beet	Genesee	July 2006
R70[c]	table beet	Genesee	July 2006
R75	snap bean	Ontario	July 2006
R77	snap bean	Ontario	July 2006
R81	table beet	Livingston	August 2006
R82	table beet	Livingston	August 2006
R83	table beet	Livingston	August 2006
R84	table beet	Genesee	August 2006
R85	table beet	Genesee	August 2006
R88	table beet	Genesee	August 2006
R89	table beet	Genesee	August 2006
R90	table beet	Genesee	August 2006
R92	snap bean	Genesee	August 2006
R93	snap bean	Genesee	August 2006
R94	snap bean	Genesee	August 2006
R100	snap bean	Genesee	August 2006
R101	snap bean	Genesee	August 2006
R104	cabbage	Ontario	August 2006
R105	snap bean	Ontario	July 2006
R106	carrot	Orleans	August 2006
R107	cabbage	Orleans	August 2006
R108	cabbage	Orleans	August 2006
R110	table beet	Genesee	August 2006
R112	table beet	Genesee	August 2006
R113	table beet	Genesee	August 2006
R115	carrot	Yates	September 2006

a) Refers to the New York county where the vegetable field was located.
(CNY = Central New York)

b) Isolate was recovered from a field planted to table beet in 2005.

c) Isolate was recovered from a field planted to corn in 2005.

Molecular Characterization

Individual isolates were grown on PDA at 27 °C and DNA was extracted using the Ultraclean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). The rDNA ITS region consisting of ITS 1, 5.8S, and ITS 2 was amplified using the polymerase chain reaction (PCR) with primers ITS4 and ITS5 for 46 isolates and ITS1 and ITS4 for 22 isolates (Table 2.2) (White et al. 1990). Reactions for PCR amplifications were performed in a 50 µL mixture containing 50 – 100 ng of template DNA, 0.2 µM of each primer, 0.2 mM of each of the four dNTPs, 1.5 units of *Taq* DNA Polymerase (New England BioLabs, Inc., Ipswich, MA), and 1x ThermoPol Buffer containing 10 mM KCl, 20 mM Tris-HCl, and 2 mM $MgSO_4$ (New England BioLabs, Inc.). The amplifications were performed with a PTC-100™ Peltier Thermal Cycler (MJ Research Inc., Waltham, MA). Cycle parameters were an initial denaturation at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. A 4 µL aliquot of each PCR product was run electrophoretically on a 1% agarose gel at 100V to confirm amplification. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). The ITS region was sequenced at the Life Sciences Core Laboratories Center of Cornell University using Big Dye Terminator chemistry and the AmpliTaq-FS DNA Polymerase on the Automated 3730 DNA Analyzer (Table 2.2) (Applied Biosystems, Foster City, CA).

Sequencing results of many isolates exhibited overlapping peaks in the fluorescent peak trace chromatograms of DNA sequence data. For such isolates PCR products were cloned using the TOPO® TA Cloning Kit for Sequencing with One Shot® TOP10 Chemically Competent *E. coli* (Invitrogen™, Carlsbad, CA). Insertion of the ITS region was confirmed by whole-cell PCR. Reactions for PCR

Table 2.2
Polymerase chain reaction (PCR) primer combinations, cloning status (Y = cloned, N = not cloned), sequencing primer(s), and GenBank accession numbers for individual isolates used in the molecular characterization.

Isolate	PCR primers	Cloned	Sequencing primers	GenBank accession No.
R1	ITS4 - ITS5	Y	ITS4, ITS5	EU591747
R2	ITS1 - ITS4	N	ITS1	NA
R3	ITS1 - ITS4	N	ITS1	EU591748
R4	ITS1 - ITS4	N	ITS4	EU591749
R5	ITS4 - ITS5	Y	ITS4, ITS5	EU591750
R6	ITS4 - ITS5	Y	ITS4, ITS5	EU591751
R7	ITS1 - ITS4	N	ITS1	EU591752
R8	ITS4 - ITS5	Y	ITS4, ITS5	EU591753
R9	ITS1 - ITS4	N	ITS1	EU591754
R10	ITS1 - ITS4	N	ITS1	EU591755
R11	ITS1 - ITS4	N	ITS1	EU591756
R12	ITS1 - ITS4	N	ITS1	EU591757
R13	ITS4 - ITS5	Y	ITS4, ITS5	EU591758
R14	ITS4 - ITS5	N	ITS4	EU591759
R15	ITS4 - ITS5	Y	ITS4, ITS5	EU591760
R16	ITS4 - ITS5	Y	ITS4, ITS5	EU591761
R17	ITS4 - ITS5	Y	ITS4, ITS5	EU591762
R18	ITS4 - ITS5	Y	ITS4, ITS5	EU591763
R20	ITS4 - ITS5	Y	ITS4, ITS5	EU591764
R21	ITS1 - ITS4	N	ITS1	EU591765
R22	ITS1 - ITS4	N	ITS1	EU591766
R25	ITS4 - ITS5	Y	ITS4, ITS5	EU591767
R27	ITS4 - ITS5	Y	ITS4, ITS5	EU591768
R29	ITS4 - ITS5	Y	ITS4, ITS5	EU591769
R31	ITS1 - ITS4	N	ITS1	EU591770
R32	ITS1 - ITS4	N	ITS4	EU591771
R33	ITS1 - ITS4	N	ITS1	EU591772
R35	ITS1 - ITS4	N	ITS1	EU591773
R36	ITS4 - ITS5	Y	ITS4, ITS5	EU591774
R37	ITS4 - ITS5	N	ITS4	EU591775
R39	ITS4 - ITS5	Y	ITS4, ITS5	EU591776
R41	ITS4 - ITS5	Y	ITS4, ITS5	EU591777
R43	ITS4 - ITS5	Y	ITS4, ITS5	EU591778
R47	ITS4 - ITS5	Y	ITS4, ITS5	EU591779

Isolate	PCR primers	Cloned	Sequencing primers	GenBank accession No.
R55	ITS1 - ITS4	N	ITS4	EU591780
R57	ITS4 - ITS5	Y	ITS4, ITS5	EU591781
R59	ITS1 - ITS4	N	ITS4	EU591782
R60	ITS4 - ITS5	N	ITS4	NA
R62	ITS4 - ITS5	N	ITS4	EU591783
R64	ITS4 - ITS5	Y	ITS4, ITS5	EU591784
R65	ITS4 - ITS5	Y	ITS4, ITS5	EU591785
R66	ITS4 - ITS5	Y	ITS4, ITS5	EU591786
R68	ITS4 - ITS5	Y	ITS4, ITS5	EU591787
R70	ITS4 - ITS5	Y	ITS4, ITS5	EU591788
R75	ITS4 - ITS5	Y	ITS4, ITS5	EU591789
R77	ITS1 - ITS4	N	ITS4	EU591790
R81	ITS4 - ITS5	Y	ITS4, ITS5	EU591791
R82	ITS1 - ITS4	N	ITS4	EU591792
R83	ITS4 - ITS5	Y	ITS4, ITS5	EU591793
R84	ITS4 - ITS5	Y	ITS4, ITS5	EU591794
R85	ITS4 - ITS5	Y	ITS4, ITS5	EU591795
R88	ITS4 - ITS5	Y	ITS4, ITS5	EU591796
R89	ITS4 - ITS5	Y	ITS4, ITS5	EU591797
R90	ITS4 - ITS5	Y	ITS4, ITS5	EU591798
R92	ITS1 - ITS4	N	ITS4	EU591799
R93	ITS4 - ITS5	N	ITS4, ITS5	EU591800
R94	ITS4 - ITS5	Y	ITS4, ITS5	EU591801
R100	ITS4 - ITS5	Y	ITS4, ITS5	EU591802
R101	ITS4 - ITS5	Y	ITS4, ITS5	EU591803
R104	ITS4 - ITS5	Y	ITS4, ITS5	EU591804
R105	ITS4 - ITS5	Y	ITS4, ITS5	EU591805
R106	ITS1 - ITS4	N	ITS4	EU591806
R107	ITS4 - ITS5	Y	ITS4, ITS5	EU591807
R108	ITS1 - ITS4	N	ITS4	EU591808
R110	ITS1 - ITS4	N	ITS4	EU591809
R112	ITS4 - ITS5	Y	ITS4, ITS5	EU591810
R113	ITS4 - ITS5	N	ITS4, ITS5	EU591811
R115	ITS4 - ITS5	Y	ITS4, ITS5	EU591812

amplifications were performed in a 25 µL mixture containing a toothpick scrape of overnight culture, 0.2 µM of each primer, 0.2 mM of each of the four dNTPs, 0.75 units of *Taq* DNA Polymerase (New England BioLabs, Inc.), and 1x ThermoPol Reaction Buffer containing 10 mM KCl, 20 mM Tris-HCl, and 2 mM $MgSO_4$ (New England BioLabs, Inc.). The amplifications were performed with the PTC-100™ Peltier Thermal Cycler (MJ Research Inc.). Cycle parameters were an initial denaturation at 95 °C for 4 min, followed by 35 cycles consisting of denaturation at 94 °C for 40 sec, annealing at 60 °C for 30 sec, extension at 72 °C for 60 sec, and a final extension at 72 °C for 10 min. A 4 µL aliquot of each PCR product was run electrophoretically on a 1% agarose gel at 100V. For each isolate, one plasmid with confirmed insertion was grown overnight in LB broth medium containing ampicillin and purified using the Wizard® *Plus* Minipreps DNA Purification System (Promega, Madison, WI). DNA concentration was quantified using the ND-1000 Spectrophotometer (Nanodrop Technologies LLC, Wilmington, DE) and sent to the sequencing facility at Cornell University. The clones were sequenced using both ITS4 and ITS5 primers (Table 2.2) (White et al. 1990).

Phylogenetic Analysis

Sequence data of complementary strands were checked and edited using SeqEd (Applied Biosystems, Foster City, CA). To infer species, AGs, and subgroups; reference sequences and the outgroup sequence (*Athelia rolfsii*) from the study conducted by Sharon et al. (2006) were obtained from GenBank (Table 2.3). Additional sequences for AG 13, AG BI, and *Ceratobasidium* AGs, CAG 1 through CAG 7, were added (Table 2.3) (Carling et al. 2002c; Gonzalez et al. 2001). A reference sequence for CAG 2 was not found, therefore a sequence from AG A (Gonzalez et al. 2001) was used to represent CAG 2, because the two groups are

Table 2.3
Reference sequences used in this study to characterize *Rhizoctonia solani* and *Rhizoctonia*-like fungi isolated in New York. GenBank accession numbers of the internal transcribed spacer (ITS) sequence, their designated anastomosis group (AG)/subgroup/species, origin, and references are listed (Carling et al. 2002a; Carling et al. 2002c; Ciampi et al. 2005; Godoy-Lutz et al. 2003; Gonzalez et al. 2001; Johanson et al. 1998; Kuninaga et al. 1997; Kuninaga et al. 2000; Kuramae et al. 2003; Pope and Carter 2001; Salazar et al. 2000; Salazar et al. 1999; Sharon et al. 2006; Sharon et al. 2007; Toda et al. 2004; Toda et al. 2007).

Species/AG/ subgroup		GenBank accession no.	Origin	Reference
	AG 1-IA	AY270010	Soybean, Brazil	Ciampi et al., 2005
		AB122133	Unknown	Toda et al., 2004
	AG 1-IB	AB122139	Unknown	Toda et al., 2004
		AF308626	Bean, Dominican Republic	Godoy-Lutz et al., 2003
	AG 1-IC	AB122142	Unknown	Toda et al., 2004
		U19951	Unknown, France	Salazar et al., 1999
	AG 1-ID	AB122128	Unknown	Toda et al., 2004
		AB122130	Unknown	Toda et al., 2004
	AG 2-1	U57729	*Pinus*, Canada	Salazar et al., 1999
		AY154317	Unknown	Kuramae et al., 2003
	AG 2-1-2t	AB054850	Tulip, Netherlands	Carling et al., 2002b
		AB054852	Tulip, Netherlands	Carling et al., 2002b
	AG 2-2LP	AB054866	Zoysia grass, Japan	Carling et al., 2002b
		AJ238163	Zoysia grass, Japan	Salazar et al., 2000
		AJ238160	Zoysia grass, Japan	Salazar et al., 2000
	AG 2-2IIIB	AF354116	Matrush, Japan	Gonzalez et al., 2001
		AJ238166	Maize, Japan	Salazar et al., 2000
	AG 2-2IV	AB000014	Sugar beet, Japan	Kuninaga et al., 1997
		AY270014	Unknown	Ciampi et al., 2005*
	AG 2-3	U57740	Soybean, Japan	Salazar et al., 1999
		AB054871	Soybean, Japan	Carling et al, 2002b
Rhizoctonia solani	AG 2-4	AB054878	Maize, USA	Carling et al., 2002b
		AB054879	Carrot, USA	Carling et al., 2002b
		AB054880	Carrot, USA	Carling et al., 2002b
	AG 2-BI	AB054873	Soil, Japan	Carling et al., 2002b
		AB054875	Soil, Japan	Carling et al., 2002b
	AG 3TB	AF153774	Tabacco, USA	Pope and Carter, 2001
		AB000004	Tabacco, USA	Kuninaga et al., 1997
	AG 3PT	AB019023	Soil, Australia	Kuninaga et al., 2000
		AB019017	Potato, USA	Kuninaga et al., 2000
	AG 4-HGI	AB000007	Spinach, Japan	Kuninaga et al., 1997
		AY152704	Tomato, Brazil	Kuramae et al., 2003
	AG 4-HGII	AB000006	Sugar beet, Japan	Kuninaga et al., 1997
		AY154308	Unknown	Kuramae et al., 2003
	AG 4-HGIII	AY154659	Spinach, Brazil	Kuramae et al., 2003
		DQ102449	Soil, Isreal	Sharon et al., 2007
	AG 5	AF153778	Unknown	Pope and Carter, 2001
		AF354113	Sugar beet, Japan	Gonzalez et al., 2001
	AG 6-HGI	AB000019	Soil, Japan	Kuninaga et al., 1997
		AF354102	Soil, Japan	Gonzalez et al., 2001
	AG 6-GV1	AF153780	Unknown, Japan	Pope and Carter, 2001
		AF153782	Soil, Japan	Pope and Carter, 2001
	AG 6-GV2	AY154304	Unknown	Kuramae et al., 2003
		AF354104	Soil, Japan	Gonzalez et al., 2001
	AG 6-GV3	AF153788	Wheat, South Africa	Pope and Carter, 2001
		AF153790	Wheat, Tanzania	Pope and Carter, 2001

Table 2.3 continued

Species/AG/ subgroup		GenBank accession no.	Origin	Reference
Rhizoctonia solani	AG 6-GV4	AF153785	Leaf litter, Australia	Pope and Carter, 2001
		AF153787	Leaf litter, Australia	Pope and Carter, 2001
	AG 7	AF354100	Soil, USA	Gonzalez et al., 2001
		AB000003	Soil, Japan	Kuninaga et al., 1997
	AG 8	AF354068	Barley, Australia	Gonzalez et al., 2001
		AF153797	Soil, Australia	Pope and Carter, 2001
		AB000011	Wheat, Australia	Kuninaga et al., 1997
	AG 9	AF354108	Potato, USA	Gonzalez et al., 2001
		AF354065	Potato, USA	Gonzalez et al., 2001
	AG 10	AF354071	Barley, Australia	Gonzalez et al., 2001
		AF153800	Soil, Australia	Pope and Carter, 2001
	AG 11	AF153802	Lupine, Australia	Pope and Carter, 2001
		AY154313	Unknown	Kuramae et al., 2003
	AG 12	AF153804	*Pterostylis acuminata*	Pope and Carter, 2001
		AF153805	*Pterostylis acuminata*	Pope and Carter, 2001
	AG 13	AB275645	Cotton, USA	Carling et al., 2002a
		AB275642	Cotton, USA	Carling et al., 2002a
	AG BI	AF354110	Soil, Japan	Gonzalez et al., 2001
		AB000044	Soil, Japan	Kuninaga et al., 1997
Waitea circinata	*W. circinata* var. *zeae*	AB213594	Soil, Japan	Toda et al., 2007
		AB213597	Unknown	Toda et al., 2007
	W. circinata var. *agrostis*	AB213575	Creeping bentgrass, Japan	Toda et al., 2007
		AB213577	Kentucky bluegrass, Japan	Toda et al., 2007
	W. circinata var. *circinata*	AB213582	Creeping bentgrass, Japan	Toda et al., 2007
		AB213581	Unknown	Toda et al., 2007
	W. circinata var. *oryzae*	AJ000195	Rice, Japan	Johanson et al., 1998
		AB213589	Rice, Japan	Toda et al., 2007
Ceratobasidium spp.	CAG 1	AF354086	Turfgrass, USA	Gonzalez et al., 2001
	AG A (CAG 2)	AF354092	Soil, Japan	Gonzalez et al., 2001
	CAG 3	AF354080	Peanut, USA	Gonzalez et al., 2001
	CAG 4	AF354081	Soybean, USA	Gonzalez et al., 2001
	CAG 5	AF354082	Cucumber, USA	Gonzalez et al., 2001
	CAG 6	AF354083	*Erigeron*, USA	Gonzalez et al., 2001
	CAG 7	AF354084	*Pittosporum*, USA	Gonzalez et al., 2001
Athelia rolfsii	*A. rolfsii*	AY684917	Unknown	Unpublished

* The GenBank accession number is not listed in the publication but inferred by GenBank.

known to be equivalent between the Japanese and American AGs (Table 2.3) (Garcia et al. 2006). Sequences of collected isolates and reference sequences were aligned using Clustal X (1.81) (Thompson et al. 1997) and its default parameters. Sequence alignment was adjusted manually by visual examination using MacClade 4.08 (Maddison and Maddison 2005).

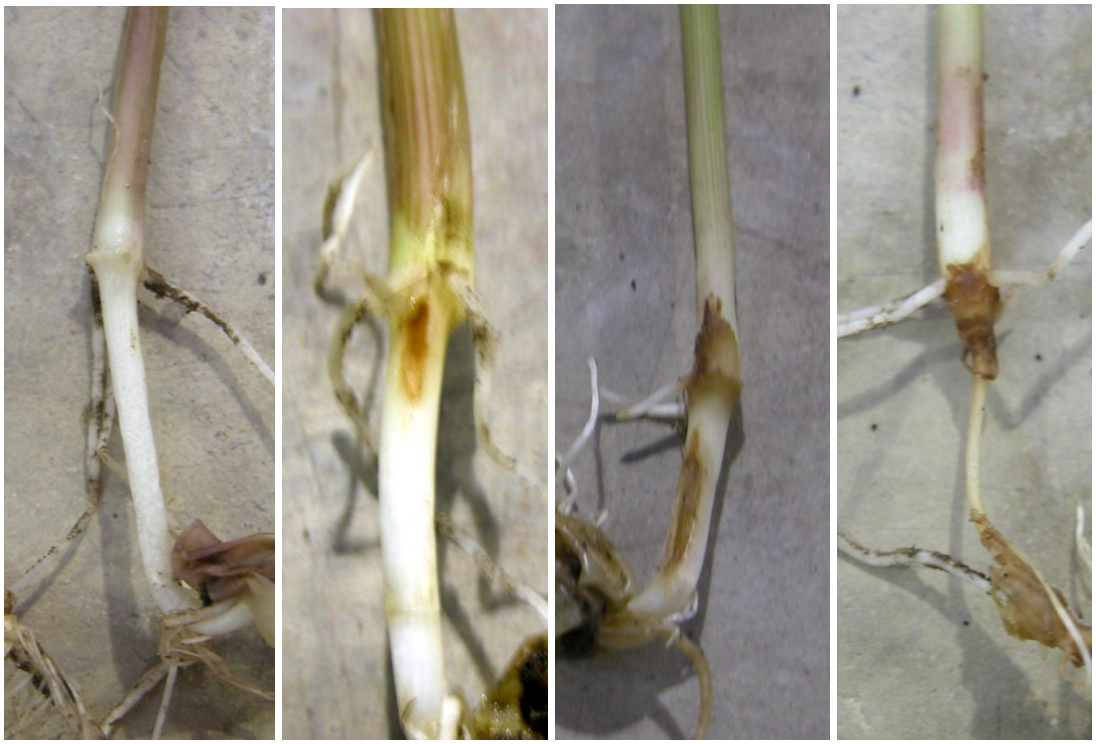
The sequence data set was analyzed using two analyses, neighbor-joining and Bayesian inference. For neighbor-joining analysis, the appropriate distance model was chosen according to the guidelines described in the *MEGA* version 4 manual (Tamura et al. 2007) based on calculating pair-wise distances (d) using the Jukes-Cantor distance model (Jukes and Cantor 1969) and the transition/transversion ratio (R). Since $d < 0.3$ ($0.00 < d < 0.272$) and R was low ($R = 1.164$), the Jukes-Cantor distance model (Jukes and Cantor 1969) was selected. A neighbor-joining tree (Saitou and Nei 1987) was generated using PAUP* ver. 4.0b10 (Swofford 2004) with bootstrap values based on 1000 replicates. For Bayesian inference, the Hasegawa-Kishino-Yano plus Gamma (HKY+G) model (Hasegawa et al. 1985) was chosen as the appropriate evolutionary model by Modeltest 3.7 (Posada and Crandall 1998) using the Akaike Information Criterion (Akaike 1974). The output parameters (number of substitution types = 2, among-site rate variation = gamma, trasition/transversion rate ratio = 1.27, state frequencies (A, C, G, T) = (0.27, 0.21, 0.16, 0.36), proportion of invariable sites = 0, gamma shape = 0.35) were entered into MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). Two million generations were run and trees were sampled every 100 generations. The first 2000 trees were discarded as the burn-in and a strict consensus tree was generated. Both neighbor-joining and Bayesian inference analyses included 833 characters and were unrooted. *A. rolfsii* was positioned as the outgroup after constructing the trees.

Pathogenicity Evaluation on Corn in the Greenhouse

To determine whether the representative isolates from New York were capable of causing disease on corn, a series of greenhouse bioassays were performed. Untreated sweet white corn cultivar Silver Princess was planted in 3.8 x 21 cm cone-tubes (Stuewe & Sons, Inc). Approximately 200 cm^3 of pasteurized soil (60 °C for 30 min) was placed in each tube and planted with two seeds of corn. Inoculum of selected collected isolates was prepared by growing isolates on PDA for 5-7 days. Seven or eight days after planting, the soil adjacent to the stem of corn seedlings was removed and a colonized PDA disk (8 mm in diameter) was placed in the pocket against the lower stem (Figure 2.1). Non-colonized PDA disks were placed next to the stems of the negative controls. After inoculation, the disks were covered with soil to prevent drying. An individual tube represents a replication and each trial consisted of four replications per isolate. Inoculation trials were conducted at four time-points; two trials were conducted for each isolate on different dates and all four trials included negative controls; thus with a total of 16 observations for each isolate and 32 observations for the negative control. After two weeks of incubation (Figure 2.2), plants were carefully removed from the soil and the roots were washed. Disease severity was assessed using a rating scale from 0 to 5; where 0 = no symptoms observed (healthy), 1 = lesions smaller than 2 mm, 2 = lesions larger than 2 mm, 3 = girdling lesion on crown tissue, 4 = rotted mesocotyl causing wire-stem symptoms, and 5 = dead seedling. Examples of symptoms for the various categories are shown in Figure 2.3. After harvest, pieces of symptomatic corn tissue from several treatments were placed on acidified PDA to ascertain the presence and recovery of the same *R. solani* or *Rhizoctonia*-like fungi used in the inoculation.

Figure 2.1
Method of inoculation of corn seedlings: One week after planting, soil adjacent to the lower stem was removed and colonized potato dextrose agar (PDA) disks were place in the pocket. The disks were then covered with soil to prevent drying.

Figure 2.2
Incubation of corn seedlings in the greenhouse for 2 weeks after inoculation.



0 **2** **3** **4**

Figure 2.3
Representation of the disease rating scale (0 to 5) used to assess disease severity on corn caused by *Rhizoctonia solani* and *Rhizoctonia*-like fungi tested in this study: 0 = no symptoms, 1 = lesions smaller than 2 mm, 2 = lesions larger than 2 mm, 3 = girdling lesions on crown tissue, 4 = rotted mesocotyl tissue with wire-stem symptoms, and 5 = dead seedlings. Pictures are from corn seedlings that were in the actual experiments.

Pathogenicity Evaluation on Snap Beans in the Greenhouse

This preliminary experiment was conducted to compare the virulence of the collected *R. solani* and *Rhizoctonia*-like isolates on corn to that on vegetables. The snap bean cultivar Hystyle was used as a model for vegetables to contrast the virulence of selected isolates in the greenhouse. Seven isolates (R18, R20, R25, R31, R39, R47, and R62) that showed variable virulence on corn were chosen to inoculate snap bean plants. Seeds of snap bean cultivar Hystyle treated with Captan®, Maxim®, and Thiram® were planted in 10 cm diameter clay pots (4 seeds per pot) filled with pasteurized soil. Inoculum of the collected isolates was prepared by growing the isolates on PDA for 10 days. Eleven days after planting, snap bean seedlings were inoculated in the same way the corn seedlings were inoculated as mentioned above. After inoculation, the disks were covered with soil to prevent drying. An individual pot represented a replication and there were four replications per isolate. The trial was conducted only once. After two weeks of incubation, plants were carefully removed from the soil and the roots and stems were washed. Disease severity was assessed using a rating scale of 0 to 3; where 0 = no symptoms (healthy), 1 = superficial lesions, 2 = sunken distinct lesions, 3 = rotted lower hypocotyl tissues showing initial symptoms of wire-stem. Examples of symptoms for the various disease categories are shown in Figure 2.4.

Statistical Analysis

Data from both corn and snap bean experiments were analyzed using SAS® software Version 9 (SAS Institute Inc., Cary, NC). The ordinal data did not have a normal distribution, therefore they were analyzed using the non-parametric methodology of Brunner and colleagues (Brunner et al. 2002) as described by (Shah and Madden 2004). For analyses on corn, data were pooled from the two trials to

0 **1** **2** **3**

Figure 2.4
Representation of the disease rating scale (0 to 3) used to assess disease severity on snap beans caused by *Rhizoctonia solani* and *Rhizoctonia*-like fungi tested in this study: 0 = no symptoms, 1 = superficial lesions, 2 = sunken distinct lesions, 3 = rotted hypocotyl tissues with initial symptoms of wire-stem. Pictures are from snap bean seedlings that were in the actual experiments.

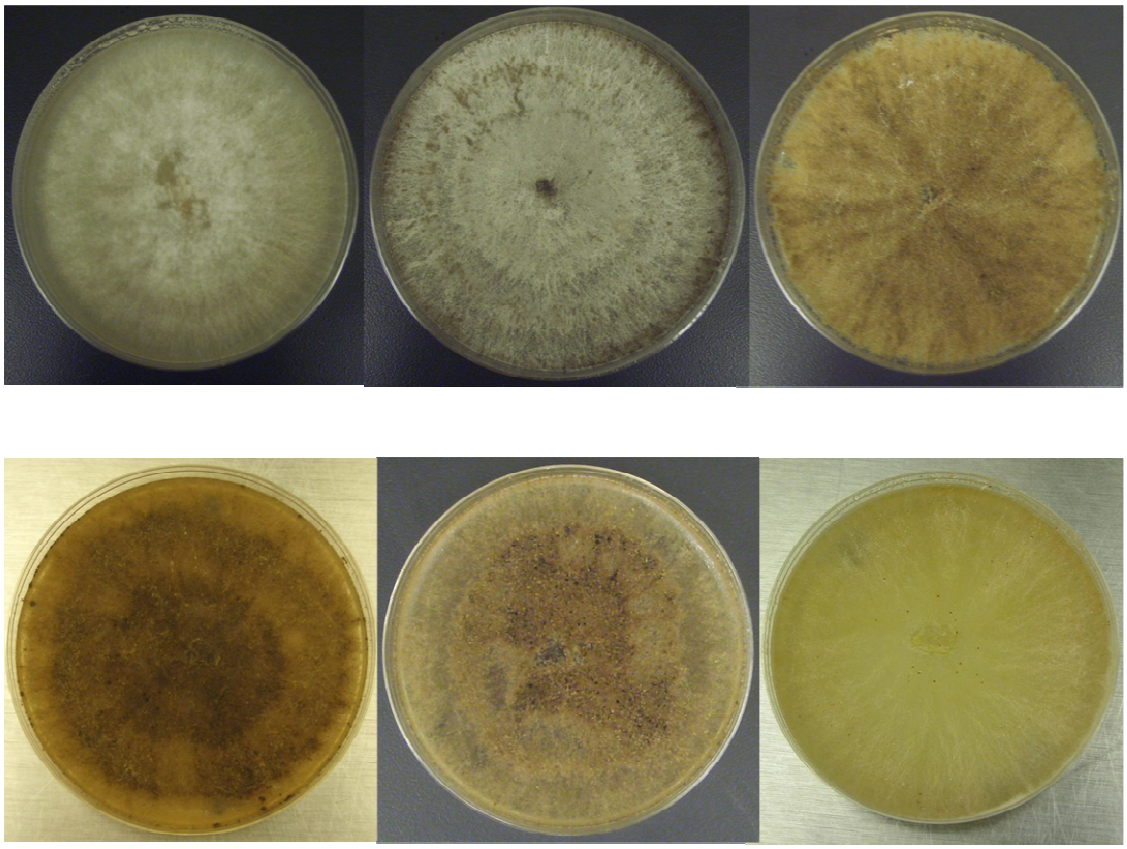


Figure 2.5
A subset of *Rhizoctonia solani* and *Rhizoctonia*-like fungi collected in New York to show their morphological diversity. Isolates were grown on PDA for 2 weeks.

obtain overall results for analysis on corn. Two analyses were performed; the first analysis assessed disease severity caused by individual isolates and the second analysis assessed disease severity in relation to the inferred identification of isolates. For analysis on snap bean, the incidence of infection on snap bean was relatively low, therefore only infected plants were included in the analysis.

Results

Phylogenetic analysis

In the neighbor-joining tree, collected isolates were inferred to belong to an AG, subgroup, or species if they formed a cluster including a reference sequence supported by a bootstrap value (BS) of 95% or higher, and were considered closely related to a group if supported by a BS lower than 95%. The analysis inferred the identification of 61 isolates: Twenty-six isolates (38%) were AG 2-2, 19 isolates (28 %) were AG 4, 6 isolates (9%) were AG 1, 3 isolates (4%) were AG 2-1, 2 isolates (3%) were AG 5, 2 isolates were *W. circinata* var. *zeae*, 1 isolate (1%) was AG 11, 1 isolate (1%) was CAG 2, and 1 (1%) isolate was CAG 6. The remaining 7 isolates were closely related to CAG 2 supported by a BS 87% (Figure 2.6). As for identifying isolates to further subgroup of cultural type, within AG 2-2; 10 isolates belonged to AG 2-2IV, within AG 4; 1 isolate belonged to AG 4-HGI and 15 isolates were closely related to AG 4-HGII (BS 81%), and within AG 1; 4 isolates belonged to AG 1-IC and 2 isolates belonged to AG 1-IB (Figure 2.6). Remaining isolates within AG 2-2 and AG 4 could not be identified to further subgroup or cultural type.

In the Bayesian inference tree, collected isolates were inferred to belong to an AG, subgroup, or species if they formed a cluster including a reference sequence supported by a posterior probability value (PP) of 90% or higher and were considered closely related to a group if supported by a PP lower than 90%. Using this criterion,

Figure 2.6
Neighbor-joining tree generated from the rDNA ITS1-5.8S- ITS2 region of the nuclear ribosomal DNA of reference sequences and collected New York isolates. Bootstrap values are based on 1000 replicates and values greater than 50% are indicated in italics. Reference sequences are indicated with GenBank accession numbers followed by the species, AG, or subgroup designation and New York isolates are indicated by the isolate number following the letter R. Inferred identifications are indicated on the right (*W. c.* v. *zeae* = *Waitea circinata* var. *zeae*). Host of origin of collected New York isolates are indicated after the isolate number: CG = cabbage, CT = carrot, DB = dry bean, P = pea, SB = snap bean, TB = table beet.


AG 4
AG 1
AG 2-1
AG 2-2
AG 11
AG 5
CAG 6
CAG 2
W. c. v. zeae
AY154659 4 HGIII
DQ102449 4 HGIII
AB000007 4 HGI
AY152704 4 HGI
AY154308 4 HGII
AB000006 4 HGII
AF354086 CAG1
AF354092 A CAG2
AF354084 CAG7
AF354081 CAG4
AF354083 CAG6
AF354082 CAG5
AF354080 CAG3
AF354100 7
AB000003 7
AF153778 5
AF354113 5
AF153802 11
AF354116 2 2IIIB
AJ238166 2 2IIIB
AY154313 11
U57740 2 3
AB054871 2 3
AJ238160 2 2LP
AJ238163 2 2LP
AB054866 2 2LP
AY2700014 2 2IV
AJ238164 2 2IV
AB000014 2 2IV
AB019017 3PT
AB019023 3PT
AF153774 3TB
AB000004 3TB
AF153600 10
AF354071 10
AB054879 2 4
AB054880 2 4
AB054878 2 4
AF354065 9
AF354108 9
AY154317 2 1
AB054852 2 1 2I
AB054850 2 1 2I
U57729 2 1
AB054873 2 BI
AF354110 BI
AB054875 2 BI
AB000044 BI
AF354102 6 HGI
AB000019 6 HGI
AF153782 6 GV1
AF153780 6 GV1
AF354104 6 GV2
AY154304 6 GV2
AF153787 6 GV4
AF153785 6 GV4
AF153788 6 GV3
AF153790 6 GV3
AF153804 12
AF153805 12
AB275645 13
AB275642 13
AB122142 1 IC
UT9951 1 IC
AB122139 1 IB
AF308626 1 IB
AB122130 1 ID
AB122128 1 ID
AY270010 1 IA
AB122133 1 IA
AB213594 ZEAE
AB213597 ZEAE
AB213575 AGROSTIS
AB213577 AGROSTIS
AB213589 ORYZAE
AJ000195 ORYZAE
AB213582 CIRCINATA
AB213581 CIRCINATA
AY684917 AR
0.005 substitutions/site

Bayesian analysis (Figure 2.7) supported the inferences suggested by the neighbor-joining analysis for 61 isolates. As for the remaining 7 isolates; 5 isolates were closely related to CAG 2 (PP 38%) and the identity of 2 isolates (R20 and R59) could not be determined. In comparison to the neighbor-joining analysis, the Bayesian tree was better resolved and permitted tentative inferences about subgroups and cultural types. Within AG 2-2; 5 isolates belonged to AG 2-2IIIB, 1 isolate was closely related to AG 2-2IIIB (PP 86%), 16 isolates were closely related to AG 2-2IV (PP 80% and PP 64%), and 4 isolates were closely related to AG 2-2LP (PP 84%). Within AG 4; 1 isolate belonged to AG 4-HGI, 1 isolate was closely related to AG 4-HGI (PP 77%), 15 isolates belonged to AG 4-HGII, and 1 isolate was closely related to AG 4-HGII (PP 58%). Within AG 1; 2 isolates belonged to AG 1-IB and 4 isolates belonged to AG 1-IC (Figure 2.7).

Pathogenicity Evaluation on Corn in the Greenhouse

Disease severity on corn caused by *R. solani* and *Rhizoctonia*-like fungi isolated in New York was evaluated using relative treatment effects and their 95% confidence intervals (CI) (Table 2.4). Among the 32 observations of the negative control, 2 plants were minimally infected with a disease rating of 1. This was potentially due to contamination through splashing of soil during watering. Sixty-one isolates were considered pathogenic to corn, whereas pathogenicity of the remaining 7 isolates (R20, R62, R60, R65, R18, R59, and R36) could not be confirmed as it was within the range of the chance of contamination. There were significant differences in disease severity on corn caused by individual isolates (Table 2.4). In addition, there were significant differences in disease severity on corn when the isolates were grouped according to inferred AG/subgroup/species of *R. solani* and *Rhizoctonia*-like

Figure 2.7
Bayesian inference tree generated from the rDNA ITS1-5.8S- ITS2 region of the nuclear ribosomal DNA of reference sequences and collected isolates. Bayesian posterior probability values greater than 50% are indicated in italics. Reference sequences are indicated with GenBank accession numbers followed by the species, AG, or subgroup designation and New York isolates are indicated by the isolate number following the letter R. Inferred identifications are indicated on the right (*W. c.* v. *zeae* = *Waitea circinata* var. *zeae*). Host of origin of collected New York isolates are indicated after the isolate number: CG = cabbage, CT = carrot, DB = dry bean, P = pea, SB = snap bean, TB = table beet.

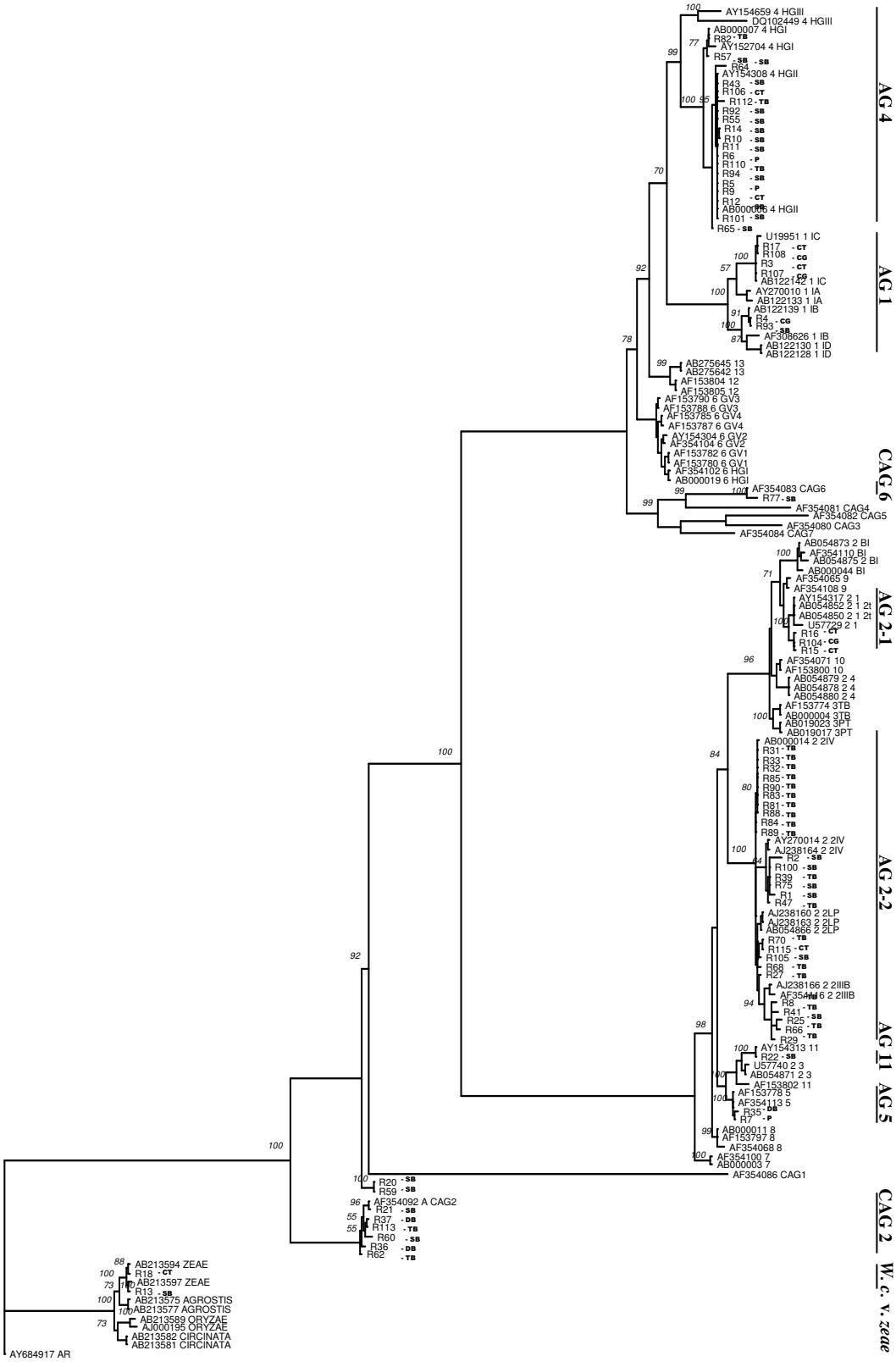

AG 4
AG 1
CAG 6
AG 2-1
AG 2-2
AG 11
AG 5
CAG 2
W. c. v. zeae
AY154659 4 HGIII
DQ102449 4 HGIII
AB000007 4 HGI
AY152704 4 HGI
AY154308 4 HGII
AB000006 4 HGII
U19951 1 IC
AB122142 1 IC
AY270010 1 IA
AB122133 1 IA
AB122139 1 IB
AF308626 1 IB
AB122130 1 ID
AB122128 1 ID
AB275645 13
AB275642 13
AF153804 12
AF153805 12
AF153790 6 GV3
AF153788 6 GV3
AF153785 6 GV4
AF153787 6 GV4
AY154304 6 GV2
AF354104 6 GV2
AF153782 6 GV1
AF153780 6 GV1
AF354102 6 HGI
AB000019 6 HGI
AF354083 CAG6
AF354081 CAG4
AF354082 CAG5
AF354080 CAG3
AF354084 CAG7
AB054873 2 BI
AF354119 BI
AB054875 2 BI
AB000044 BI
AF354065 9
AF354108 9
AB054850 2 1 2t
U57729 2 1
AF354071 10
AF153800 10
AB054879 2 4
AB054878 2 4
AB054880 2 4
AF153774 3TB
AB000004 3TB
AB019023 3PT
AB019017 3PT
AB000014 2 2IV
AY270014 2 2IV
AJ238164 2 2IV
AJ238160 2 2LP
AJ238163 2 2LP
AB054866 2 2LP
AJ238166 2 2IIIB
AF354116 2 2IIIB
AY154313 11
U57740 2 3
AB054871 2 3
AF153802 11
AF153778 5
AF354113 5
AB000011 8
AF153797 8
AF354068 8
AF354100 7
AB000003 7
AF354086 CAG1
AF354092 A CAG2
AB213594 ZEAE
AB213597 ZEAE
AB213575 AGROSTIS
AB213577 AGROSTIS
AB213589 ORYZAE
AJ000195 ORYZAE
AB213582 CIRCINATA
AB213581 CIRCINATA
AY684917 AR
– 10 changes

Table 2.4
Median and relative treatment effects (*p*) with 95% confidence intervals (CI) of disease severity ratings obtained for individual isolates from the corn and snap bean pathogenicity trials. Isolates are ranked in ascending order of relative treatment effects from the corn pathogenicity trial. Each isolate is accompanied by the inferred identification of anasotmosis group (AG), subgroup, or species and the host of isolation. Disease severity ratings on corn were determined on a scale of 0 to 5, and those on snap bean were determined on a scale of 0 to 3. Relative treatment effects were calculated using the non-parametric method for ordinal data described by Shah and Madden (2004).

Isolate	Inferred AG	Host Plant	Corn			Snap Bean		
			Median	*p*	95% CI for *p*	Median	*p*	95% CI for *p*
Negative control	NA	NA	0	0.151	(0.131, 0.17)	0	0.269	(0.228, 0.31)
R20	CAG 2	snap bean	0	0.166	(0.111, 0.221)	1	0.516	(0.401, 0.631)
R62	CAG 2	table beet	0	0.191	(0.087, 0.295)	1	0.487	(0.376, 0.599)
R60	CAG 2	snap bean	0	0.207	(0.131, 0.283)			
R65	AG 4	snap bean	0	0.207	(0.131, 0.283)			
R18	*W. circinata* var. *zeae*	carrot	0	0.22	(0.142, 0.297)	1	0.419	(0.313, 0.525)
R59	CAG 2	snap bean	0	0.22	(0.142, 0.297)			
R36	CAG 2	dry bean	0	0.26	(0.163, 0.357)			
R37	CAG 2	dry bean	0	0.26	(0.173, 0.348)			
R15	AG 2-1	carrot	0	0.273	(0.187, 0.36)			
R16	AG 2-1	carrot	0	0.291	(0.188, 0.393)			
R9	AG 4	carrot	0.5	0.301	(0.209, 0.394)			
R77	CAG 6	snap bean	0.5	0.301	(0.209, 0.394)			
R113	CAG 2	table beet	0.5	0.308	(0.197, 0.418)			
R14	AG 4	snap bean	1	0.312	(0.231, 0.393)			
R104	AG 2-1	cabbage	0.5	0.316	(0.218, 0.415)			
R12	AG 4	snap bean	1	0.329	(0.233, 0.425)			
R112	AG 4	table beet	0.5	0.332	(0.228, 0.435)			
R94	AG 4	snap bean	0	0.344	(0.213, 0.474)			
R21	CAG 2	snap bean	1	0.372	(0.271, 0.473)			
R110	AG 4	table beet	1	0.39	(0.257, 0.523)			
R5	AG 4	pea	1	0.393	(0.282, 0.503)			
R55	AG 4	snap bean	1.5	0.413	(0.319, 0.507)			
R92	AG 4	snap bean	1	0.424	(0.345, 0.503)			
R107	AG 1	cabbage	2	0.452	(0.334, 0.571)			
R108	AG 1	cabbage	2	0.452	(0.334, 0.571)			
R105	AG 2-2	snap bean	2	0.462	(0.328, 0.597)			
R115	AG 2-2	carrot	1	0.468	(0.33, 0.605)			
R6	AG 4	pea	1.5	0.477	(0.381, 0.573)			
R17	AG 1	carrot	2	0.478	(0.372, 0.584)			
R106	AG 4	carrot	2	0.478	(0.372, 0.584)			
R43	AG 4	snap bean	2	0.484	(0.403, 0.565)			
R83	AG 2-2	table beet	1.5	0.491	(0.33, 0.651)			
R4	AG 1	cabbage	2	0.525	(0.471, 0.579)			
R13	*W. circinata* var. *zeae*	snap bean	2	0.54	(0.443, 0.636)			

Table 2.4 continued

Isolate	Inferred AG	Host Plant	Corn			Snap Bean		
			Median	*p*	95% CI for *p*	Median	*p*	95% CI for *p*
R101	AG 4	snap bean	2	0.552	(0.467, 0.638)			
R35	AG 5	dry bean	2	0.553	(0.44, 0.667)			
R68	AG 2-2	table beet	2	0.555	(0.415, 0.695)			
R3	AG 1	carrot	2	0.558	(0.487, 0.628)			
R82	AG 4	table beet	2	0.564	(0.487, 0.642)			
R90	AG 2-2	table beet	2	0.564	(0.43, 0.697)			
R31	AG 2-2	table beet	3	0.572	(0.412, 0.732)	1	0.518	(0.389, 0.647)
R22	AG 11	snap bean	2	0.574	(0.435, 0.713)			
R70	AG 2-2	table beet	2	0.574	(0.438, 0.711)			
R10	AG 4	snap bean	2	0.59	(0.501, 0.679)			
R32	AG 2-2	table beet	2.5	0.593	(0.453, 0.734)			
R93	AG 1	snap bean	2	0.599	(0.49, 0.708)			
R57	AG 4	snap bean	2	0.606	(0.522, 0.689)			
R33	AG 2-2	table beet	2	0.617	(0.516, 0.718)			
R66	AG 2-2	table beet	2	0.631	(0.496, 0.765)			
R88	AG 2-2	table beet	2	0.633	(0.51, 0.755)			
R11	AG 4	snap bean	2	0.64	(0.536, 0.744)			
R64	AG 4	snap bean	2	0.646	(0.558, 0.733)			
R81	AG 2-2	table beet	2	0.646	(0.543, 0.748)			
R89	AG 2-2	table beet	2	0.654	(0.553, 0.755)			
R84	AG 2-2	table beet	3	0.68	(0.551, 0.809)			
R1	AG 2-2	snap bean	2.5	0.697	(0.597, 0.798)			
R7	AG 5	pea	2	0.697	(0.612, 0.783)			
R29	AG 2-2	table beet	2.5	0.717	(0.608, 0.826)			
R41	AG 2-2	table beet	3	0.727	(0.61, 0.844)			
R27	AG 2-2	table beet	2.5	0.732	(0.656, 0.808)			
R85	AG 2-2	table beet	3	0.733	(0.653, 0.814)			
R100	AG 2-2	snap bean	3	0.775	(0.699, 0.851)			
R47	AG 2-2	table beet	3	0.777	(0.674, 0.88)	1	0.505	(0.379, 0.632)
R2	AG 2-2	snap bean	3	0.785	(0.694, 0.876)			
R8	AG 2-2	table beet	3.5	0.792	(0.699, 0.884)			
R25	AG 2-2	snap bean	3	0.813	(0.739, 0.887)	1	0.518	(0.389, 0.647)
R75	AG 2-2	snap bean	3.5	0.818	(0.735, 0.901)			
R39	AG 2-2	table beet	4	0.898	(0.872, 0.924)	2	0.795	(0.703, 0.886)

fungi (Figure 2.8). AG 2-2 isolates were the most virulent, whereas CAG 2 isolates were the least virulent on corn (Figure 2.8).

Pathogenicity evaluation on snap bean

Disease severity on snap bean caused by the selected 7 isolates of *R. solani* and *Rhizoctonia*-like fungi collected in New York was evaluated using relative treatment effects and their 95% CI (Table 2.4). All isolates tested were pathogenic to snap bean. Also, there were significant differences in disease severity caused by the selected isolates on snap bean. Isolate R18 was the least virulent on snap beans, whereas isolate R39 was the most virulent (Table 2.4).

Discussion

Molecular characterization of *R. solani* and *Rhizoctonia*-like fungi in New York showed the presence of a diverse population with isolates belonging to AG 1, AG 2-2, AG 4, AG 5, AG 11, CAG 2, CAG 6, *W. circinata* var. *zeae*, and several isolates closely related to CAG 2 (Figure 2.6, Figure 2.7). The dominant groups were AG 2-2 (38%) and AG 4 (28%). As most isolates were collected from table beets and snap beans, this finding is consistent with results reported previously from New York (Galindo et al. 1982; Olaya and Abawi 1994a). This is the first report of *W. circinata* var. *zeae* isolation from vegetable tissues, specifically from naturally infested carrot and snap bean plants in western New York. There are only two other reports from Turkey where this fungus has been isolated from vegetables (Erper et al. 2005; Erper et al. 2006). It was also the first time that AG 11 (isolate R22) was isolated in this region. AG 11 reported to be pathogenic on wheat and lupine (Carling et al. 1994; Garcia et al. 2006; Sweetingham 1989), but isolate R22 was isolated from snap bean.

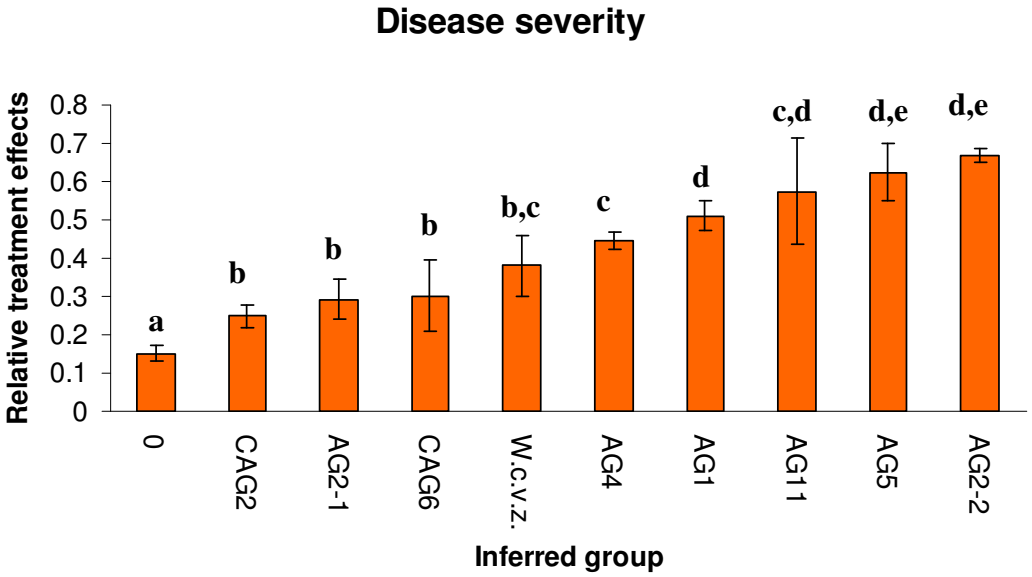


Figure 2.8
Disease severity on corn caused by *Rhizoctonia solani* and *Rhizoctonia*-like isolates collected in New York, when grouped by inferred anastomosis group (AG), subgroup, or species. Disease severity ratings were determined on a scale of 0 to 5. Relative treatment effects were calculated using the non-parametric method for ordinal data described by Shah and Madden (2004). Group 0 indicates the negative control and group W.c.v.z. indicates *W. circinata* var. *zeae*. Bars indicate 95% CI of relative treatment effects. Different letters indicate significant differences between groups using the 95% CI.

Isolates from AGs that are documented to be pathogenic on corn, AG 1, AG 2, and AG 4 were present. Within these AGs, we specifically sought the presence of subgroups/cultural types that are well-documented to be pathogenic on corn, AG 1-IA and AG 2-2IIIB, were examined. No isolates of AG 1-IA were detected; however, 6 isolates of AG 2-2IIIB were recovered in this investigation. Thus, with 6 isolates belonging to AG 2-2 IIIB, the hypothesis that subdivisions pathogenic to corn exist in New York cannot be rejected. AG 2-2 is subdivided into cultural types based on pathogenicity on mat rush, sugar beet, and warm season turf grasses: AG 2-2IIIB is designated as the rush type, AG 2-2IV is designated as the root rot type, and AG 2-2LP is designated as the large patch type (Hyakumachi et al. 1998; Ogoshi 1987). Despite the originally proposed characterization of AG 2-2 subdivisions, our results show that all three cultural types are able to infect corn and the hosts from which they were isolated, primarily snap bean and table beet. This suggests a need for a more thorough characterization criterion for AG 2-2 subdivisions.

Among the *R. solani* and *Rhizoctonia*-like fungi isolated from vegetables in New York, *R. solani* AG 2-2 isolates were most virulent on corn, AG 4 isolates were moderately virulent, and binucleate CAG 2 isolates were least virulent (Figure 2.8). These results are in agreement with those reported previously by Ithurrart, Buttner, and Petersen (2004), where an increase in disease on sugar beet was reported to occur when a narrow sugar beet-corn rotation was practiced. Most AG 2-2 isolates in this study were recovered from table beets. This explains why the earliest observations of the ineffectiveness of corn rotation in reducing severity of *Rhizoctonia* diseases on vegetables in New York were made by beet growers (Abawi, personal communication). Additionally, AG 2-2 has been confirmed to produce the sexual state, *T. cucumeris*, on table beets in commercial fields in New York (Olaya and Abawi 1994a). This suggests the possibility that AG 2-2 isolates of *R. solani* are

evolving pathogenicity traits to corn through sexual recombination. In combination with the isolation of *W. circinata* var. *zeae* from symptomatic vegetables, these findings suggest the ineffective corn rotation may be due to an expansion in host range. AG 2-2 isolates may have acquired the ability to infect corn, while *W. circinata* var. *zeae* isolates may have gained the ability to infect vegetables. Further investigation of the host range of these isolates is necessary to confirm this hypothesis. AGs or species previously considered to be non-pathogenic were not recovered in this investigation. Therefore, the emergence of new pathogenic AGs or species does not appear to be the reason behind the ineffectiveness of corn rotation reported by vegetable growers in New York.

Our results also revealed differences in AGs recovered from different hosts. Most isolates recovered from table beets belonged to AG 2-2 and most isolates recovered from cabbage belonged to AG 1. However, isolates recovered from carrot and snap bean belonged to many AGs. This suggests that certain hosts are susceptible to specific AGs, whereas other hosts are susceptible to a wide array of AGs (Figure 2.6, Figure 2.7, Table 2.4).

The preliminary pathogenicity trial on snap beans suggested a potential correlation between virulence of the collected isolates on corn and snap bean. Isolates R18, R20, R62 exhibited relatively low virulence on corn, whereas isolates R25, R31, R39, and R47 were higher in virulence. Similarly, isolates R18 and R62 were low in virulence on snap bean, whereas isolates R25, R31, and R39 exhibited higher virulence (Table 2.4). Thus, there is a correlation between the virulence on corn and snap bean for these isolates. Due to the small number of isolates that were tested for virulence on snap bean, the correlation between the virulence of the isolates on corn and snap bean should be further tested before definitive conclusions are made. If such correlation between virulence to corn and virulence to snap bean or other vegetables

can be proven with a larger number of isolates and under field conditions, it can then be recommended to avoid the use of corn as a rotation crop for snap bean or other vegetables where *R. solani* and *Rhizoctonia*-like fungi are prevalent.

In both the neighbor-joining and Bayesian trees, reference sequences of AG 2 and CAGs did not group with other reference sequences representing the same group. As for AG 2 subgroups that did not cluster together, similar results have been reported by Kuninaga et al. (1997) suggesting that subgroups within AG 2 may be phylogenetically distant and there are different opinions about whether AG 2 subgroups should be considered as distinct AGs or not (Carling 1996). Unlike other AG subgroups that are determined by pathogenicity, morphology, and biochemical properties, AG 2 subgroups are determined by hyphal fusion frequency (Carling 1996; Ogoshi 1987; Salazar et al. 1999), a criterion similar to that used in characterizing AGs. Taking this into consideration, AG 2 subgroups may be more representative of distinct AGs than subgroups and our analyses support this. As for the CAG reference sequences that did not group together, similar results have been reported by Gonzalez et al. (2001) and the taxonomy of *Ceratobasidium* and *Thanatephorus* is still under debate (Gonzalez et al. 2001). A more rigorous, multilocus approach is necessary to clarify the phylogeny of these two genera. Our analyses suggest the use of the rDNA ITS sequence in either neighbor-joining or Bayesian inference to be robust enough to identify isolates to species and AG. However, in order to classify isolates to subgroup or AG 2-2 cultural type, Bayesian inference appears to be more predictive than neighbor-joining (Figure 2.6, Figure 2.7). Sharon et al. (2006) have demonstrated that the rDNA ITS sequence cannot differentiate cultural types of AG 2-2 when used in a neighbor-joining analysis. Developing a simple approach to identify isolates among a diverse population to AG subgroups may be challenging because subgroups have been constructed under independent criteria for different AGs. For example, AG 1

subgroups are based on morphology and pathogenicity, AG 2 subgroups are based on hyphal fusion frequencies, while AG 8 subgroups are based on zymogram patterns (Carling 1996). Therefore, implementing a universal criterion to characterize AG subgroups may allow the development of a robust method to identify isolates to subgroups and subdivisions.

Our results suggest that *R. solani* and *Rhizoctonia*-like fungi that infect vegetables in western New York, especially AG 2-2 isolates, are capable of infecting corn as well. Visual observations of symptoms caused by these isolates and their re-isolation from inoculated seedlings clearly indicate that corn serves as a host plant. Isolates R66, R68, and R70 were collected from a field that was in corn rotation during 2005 and isolates R13 and R14 were collected from a field that was in table beet during 2005 (Table 2.4). The corn rotation isolates were characterized as AG 2-2 and R13 and R14 were characterized as *W. circinata* var. *zeae* and AG 4, respectively. Disease severity caused by the corn rotation isolates on corn was as high as or higher than that caused by the vegetable isolates. These findings suggest that corn should be avoided as a rotation crop especially when AG 2-2 is present in the field. Following up this study with larger sample sizes from each AG may provide a better understanding of when corn rotations should be avoided due to the presence of certain genotypes of *Rhizoctonia* in the field. In this study we looked at one sweet corn cultivar, but it will be interesting to see if certain corn cultivars are resistant to infection by *R. solani* and *Rhizoctonia*-like fungi from New York. Additionally, in contrast to the controlled environment of a greenhouse, studies should also be conducted in the field to simulate natural conditions. Future investigations to evaluate the effectiveness of other cereals as rotation crops against *R. solani* and *Rhizoctonia*-like fungi are also needed.

REFERENCES

Abawi, G. S., and S. B. Martin. 1985. *Rhizoctonia* foliar blight of cabbage in New York State. *Plant Disease* 69 (2):158-161.

Abawi, G. S., and J. W. Ludwig. 2005. Effect of three crop rotations with and without deep plowing on root rot severity and yield of beans. *Annual Report of the Bean Improvement Cooperative* 48:118-119.

Abawi, G. S., G. Olaya, and J. W. Ludwig. 1995. Occurrence of *Thanatephorus cucumeris* on snap beans in New York. *Phytopathology* 85 (12):1554.

Agrios, G. N. 2005. *Plant pathology* Fifth edition ed. New York: Elsevier Academic Press.

Akaike, H. 1974. New look at statistical-model identification. *IEEE Transactions on Automatic Control* 19 (6):716-723.

Andersen, T. F. 1996. A comparative taxonomic study of *Rhizoctonia* sensu lato employing morphological, ultrastructural and molecular methods. *Mycological Research* 100:1117-1128.

Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology* 20:329-347.

Baker, K. F. 1970. Types of *Rhizoctonia solani* diseases and their occurrence. In *Rhizoctonia solani: Biology and Pathology*, edited by J. R. J. Parmeter. Berkeley: University of California Press.

Brunner, E., S. Domhof, and F. Langer. 2002. *Nonparametric analysis of longitudinal data in factorial experiments.* New York: John Wiley & Sons.

Buddemeyer, J., B. Pfahler, J. Petersen, and B. Marlander. 2004. Genetic variation in susceptibility of maize to *Rhizoctonia solani* (AG 2-2IIIB) - symptoms and damage under field conditions in Germany. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 111 (6):521-533.

Burpee, L. L., P. L. Sanders, H. Jr Cole, and R. T. Sherwood. 1980a. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. *Mycologia* 72 (4):689-701.

Burpee, L. L., P. L. Sanders, H. Jr Cole, and R. T. Sherwood. 1980b. Pathogenicity of *Ceratobasidium cornigerum* and related fungi representing 5 anastomosis groups. *Phytopathology* 70 (9):843-846.

Carling, D. E. 1996. Grouping of *Rhizoctonia solani* by hyphal anastomosis reaction. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control*, edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Carling, D. E., S. Kuninaga, and K. A. Brainard. 2002a. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. *Phytopathology* 92 (1):43-50.

Carling, D. E., S. Kuninaga, and K. A. Brainard. 2002b. Hyphal Anastomosis Reactions, rDNA-Internal Transcribed Spacer Sequences, and Virulence Levels Among Subsets of Rhizoctonia solani Anastomosis Group-2 (AG-2) and AG-BI.

Carling, D. E., R. E. Baird, R. D. Gitaitis, K. A. Brainard, and S. Kuninaga. 2002c. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology* 92 (8):893-899.

Carling, D. E., C. S. Rothrock, G. C. Macnish, M. W. Sweetingham, K. A. Brainard, and S. W. Winters. 1994. Characterization of anastomosis group-11 (AG-11) of *Rhizoctonia solani*. *Phytopathology* 84 (12):1387-1393.

Ciampi, M. B., E. E. Kuramae, R. C. Fenille, M. C. Meyer, N. L. Souza, and P. C. Ceresini. 2005. Intraspecific evolution of *Rhizoctonia solani* AG-1 IA associated with soybean and rice in Brazil based on polymorphisms at the ITS-5.8S rDNA operon. *European Journal of Plant Pathology* 113 (2):183-196.

Cubeta, M. A., and R. Vilgalys. 1997. Population biology of the *Rhizoctonia solani* complex. *Phytopathology* 87 (4):480-484.

Donk, M. A. 1956. Notes on resupinate Hymenomycetes - II. The tulasnelloid fungi. *Reinwardtia* 3 ((3)):363-379.

Erper, I., G. Karaca, and I. Ozkoc. 2005. First report of root rot of bean and soybean caused by *Rhizoctonia zeae* in Turkey. *Plant Disease* 89 (2):203.

Erper, I., G. H. Karaca, M. Turkkan, and I. Ozkoc. 2006. Characterization and pathogenicity of *Rhizoctonia* spp. from onion in Amasya, Turkey. *Journal of Phytopathology* 154 (2):75-79.

Galindo, J. J., G. S. Abawi, and H. D. Thurston. 1982. Variability among isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York. *Plant Disease* 66 (5):390-394.

Garcia, V. G., M. A. P. Onco, and V. R. Susan. 2006. Review. Biology and systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research* 4 (1):55-79.

Godoy-Lutz, G., J. R. Steadman, B. Higgins, and K. Powers. 2003. Genetic variation among isolates of the web blight pathogen of common bean based on PCR-RFLP of the ITS-rDNA region. *Plant Disease* 87 (7):766-771.

Gonzalez, D., M. A. Cubeta, and R. Vilgalys. 2006. Phylogenetic utility of indels within ribosomal DNA and beta-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. *Molecular Phylogenetics and Evolution* 40 (2):459-470.

Gonzalez, D., D. E. Carling, S. Kuninaga, R. Vilgalys, and M. A. Cubeta. 2001. Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia* 93 (6):1138-1150.

Hasegawa, M., H. Kishino, and T. A. Yano. 1985. Dating of the human ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22 (2):160-174.

Huber, Don M., and Donald R. Sumner. 1996. Suppressive soil amendments for the control of *Rhizoctonia* species. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control*, edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Hyakumachi, M., and T. Ui. 1987. Non-self-anastomosing isolates of *Rhizoctonia solani* obtained from fields of sugar-beet monoculture. *Transactions of the British Mycological Society* 89:155-159.

Hyakumachi, M., A. Priyatmojo, M. Kubota, and H. Fukui. 2005. New anastomosis groups, AG-T and AG-U, of binucleate Rhizoctonia spp. causing root and stem rot of cut-flower and miniature roses. *Phytopathology* 95 (7):784-792.

Hyakumachi, M., T. Mushika, Y. Ogiso, T. Toda, K. Kageyama, and T. Tsuge. 1998. Characterization of a new cultural type (LP) of *Rhizoctonia solani* AG2-2 isolated from warm-season turfgrasses, and its genetic differentiation from other cultural types. *Plant Pathology (Oxford)* 47 (1):1-9.

Ithurrart, M. E. F., G. Buttner, and J. Petersen. 2004. *Rhizoctonia* root rot in sugar beet (*Beta vulgaris* ssp *altissima*) - Epidemiological aspects in relation to maize (*Zea mays*) as a host plant. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 111 (3):302-312.

Johanson, Andrea, Helen C. Turner, Gareth J. McKay, and Averil E. Brown. 1998. A PCR-based method to distinguish fungi of the rice sheath-blight complex, *Rhizoctonia solani*, *R. oryzae* and *R. oryzae-sativae*. *FEMS Microbiology Letters* 162 (2):289-294.

Johnk, Janell Stevens, and Roger K. Jones. 2001. Differentiation of three homogeneous groups of *Rhizoctonia solani* anastomosis group 4 by analysis of fatty acids. *Phytopathology* 91 (9):821-830.

Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. In *Mammalian protein metabolism* edited by H. N. Munro. New York: Academic Press.

Katan, Jaacov. 1996. Soil solarization for the control of diseases caused by *Rhizoctonia* spp. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control*, edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Kataria, H. R., and U. Gisi. 1996. Chemical control of *Rhizoctonia* species. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Dordrecht / Boston / London: Kluwer Academic Publisher.

Kuninaga, S., T. Natsuaki, T. Takeuchi, and R. Yokosawa. 1997. Sequence variation of the rDNA ITS regions within and between anastomosis groups in *Rhizoctonia solani*. *Current Genetics* 32 (3):237-243.

Kuninaga, Shiro, Donald E. Carling, Toru Takeuchi, and Ryozo Yokosawa. 2000. Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG-3. *Journal of General Plant Pathology* 66 (1):2-11.

Kuramae, E. E., A. L. Buzeto, M. B. Ciampi, and N. L. Souza. 2003. Identification of *Rhizoctonia solani* AG 1-IB in lettuce, AG 4 HG-I in tomato and melon, and AG 4 HG-III in broccoli and spinach, in Brazil. *European Journal of Plant Pathology* 109 (4):391-395.

Kuramae, E. E., A. L. Buzeto, A. K. Nakatani, and N. L. Souza. 2007. rDNA-based characterization of a new binucleate *Rhizoctonia* spp. causing root rot on kale in Brazil. *European Journal of Plant Pathology* 119 (4):469-475.

Leach, L. D., and R. H. Garber. 1970. Control of *Rhizoctonia solani*. In *Rhizoctonia solani: Biology and Pathology*, edited by J. R. J. Parmeter. Berkeley: University of California Press.

Lehtonen, M. J., P. Ahvenniemi, P. S. Wilson, M. German-Kinnari, and J. P. T. Valkonen. 2008. Biological diversity of *Rhizoctonia solani* (AG-3) in a

northern potato-cultivation environment in Finland. *Plant Pathology* 57 (1):141-151.

Li, Wu, and Yan. 1998. Aetiology of *Rhizoctonia* in sheath blight of maize in Sichuan. *Plant Pathology* 47 (1):16-21.

Maddison, W. P., and D. R. Maddison. 2005. *MacClade 4.08: Analysis of phylogeny and character evolution* Sunderland, MA: Sinauer, .

Manici, L. M., and P. Bonora. 2007. Molecular genetic variability of Italian binucleate *Rhizoctonia* spp. isolates from strawberry. *European Journal of Plant Pathology* 118 (1):31-42.

Mazzola, M., R. W. Smiley, A. D. Rovira, and R. J. Cook. 1996. Chracterization of *Rhizoctonia* isolates, disease occurence and management in cereals. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Dordrecht / Boston / London: Kluwer Academic Publisher.

McNeill, J., F. R. Barrie, H. M. Burdet, V. Demoulin, D. L. Hawksworth, K. Marhold, D. H. Nicolson, J. Prado, P. C. Silva, J. E. Skog, J. H. Wiersema, and N. J. Turland. 2006. International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress. In *International Botanical Congress*. Vienna, Austria: International Association for Plant Taxonomy

Menzies, J. D. 1970. Introduction the 1st century of *Rhizoctonia solani*. In *Rhizoctonia solani: Biology and Pathology*, edited by J. R. J. Parmeter. Berkeley: University of California Press.

Moore, Royall T. 1987. The Genera of *Rhizoctonia*-like fungi: *Ascorhizoctonia*, *Ceratorhiza gen. nov.*, *Epulorhiza gen. nov.*, *Moniliopsis,* and *Rhizoctonia*. *Mycotaxon* 29:91-99.

Moore, Royall T. 1996. The dolipore/parenthesome septum in modern taxonomy. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Naito, S. 1996. Basidiospore dispersal and survival. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Naito, Shigeo, and Seiji Kanematsu. 1994. Characterization and pathogenicity of a new anastomosis subgroup AG-2-3 of *Rhizoctonia solani* Kuhn isolated from leaves of soybean. *Annals of the Phytopathological Society of Japan* 60 (6):681-690.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. In *Annual Review of Phytopathology, Vol. 25*, edited by R. J. Cook. Palo Alto, California: Annual Reviews Inc.

Ogoshi, A. 1996. Introduction - The genus *Rhizoctonia*. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Ogoshi, A., M. Oniki, T. Araki, and T. Ui. 1983a. Anastomosis groups of binucleate *Rhizoctonia* in Japan and North America and their perfect states. *Nippon Kingakukai Kaiho* 24 (1):79-88.

Ogoshi, A., Oniki M., Araki T., and Ui T. 1983b. Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect states. *Journal of the Faculty of Agriculture Hokkaido University* 61 (2):244-260.

Olaya, G., and G. S. Abawi. 1991. Occurrence of *Thanatephorus cucumeris* on table beets in New York state. *Phytopathology* 81 (10):1186.

Olaya, G., and G. S. Abawi. 1994a. Characteristics of *Rhizoctonia solani* and binucleate *Rhizoctonia* species causing foliar blight and root rot on table beets in New York state. *Plant Disease* 78 (8):800-804.

Olaya, G., and G. S. Abawi. 1994b. Influence of inoculum type and moisture on development of *Rhizoctonia solani* on foliage of table beets. *Plant Disease* 78 (8):805-810.

Olaya, G., G. S. Abawi, and J. Barnard. 1994. Response of *Rhizoctonia solani* and binucleate *Rhizoctonia* to 5 fungicides and control of pocket rot of table beets with foliar sprays. *Plant Disease* 78 (11):1033-1037.

Oniki, M., A. Ogoshi, T. Araki, R. Sakai, and S. Tanaka. 1985. The perfect state of *Rhizoctonia oryzae* and *Rhizoctonia zeae*, and the anastomosis groups of *Waitea circinata*. *Transactions of the Mycological Society of Japan* 26 (2):189-198.

Parmeter, J. R., H. S. Whitney, and W. D. Platt. 1967. Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. *Phytopathology* 57 (2):218-223.

Pope, E. J., and D. A. Carter. 2001. Phylogenetic placement and host specificity of mycorrhizal isolates belonging to AG-6 and AG-12 in the *Rhizoctonia solani* species complex. *Mycologia* 93 (4):712-719.

Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14 (9):817-818.

Priyatmojo, Achmadi, Verma E. Escopalao, Naomi G. Tangonan, Cecilia B. Pascual, Haruhisa Suga, Koji Kageyama, and Mitsuro Hyakumachi. 2001. Characterization of a new subgroup of *Rhizoctonia solani* anastomosis group 1 (AG-1-ID), causal agent of a necrotic leaf spot on coffee. *Phytopathology* 91 (11):1054-1061.

Reiners, S., and C. H. Petzoldt, eds. 2006. *2006 Integrated crop and pest management guidelines for commercial vegetable production*. Ithaca: Cornell University

Rinehart, T. A., W. E. Copes, T. Toda, and M. A. Cubeta. 2007. Genetic characterization of binucleate *Rhizoctonia* species causing web blight on *Azalea* in Mississippi and Alabama. *Plant Disease* 91 (5):616-623.

Roberts, Peter. 1999. *Rhizoctonia-forming fungi*. Kew: Royal Botanic Gardens.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12):1572-1574.

Saitou, N., and M. Nei. 1987. The neighbor-joining method a new method for reconstructing phylogenetic trees. *Molecular Biology & Evolution* 4 (4):406-425.

Salazar, Oscar, Maria C. Julian, Mitsuro Hyakumachi, and Victor Rubio. 2000. Phylogenetic grouping of cultural types of *Rhizoctonia solani* AG 2-2 based on ribosomal ITS sequences. *Mycologia* 92 (3):505-509.

Salazar, Oscar, Johannes H. M. Schneider, Maria C. Julian, Jaap Keijer, and Victor Rubio. 1999. Phylogenetic subgrouping of *Rhizoctonia solani* AG 2 isolates based on ribosomal ITS sequences. *Mycologia* 91 (3):459-467.

Shah, D. A., and L. V. Madden. 2004. Nonparametric analysis of ordinal data in designed factorial experiments. *Phytopathology* 94 (1):33-43.

Sharon, Michal, Shiro Kuninaga, Mitsuro Hyakumachi, and Baruch Sneh. 2006. The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. *Mycoscience* 47 (6):299-316.

Sharon, Michal, Stanley Freeman, Shiro Kuninaga, and Baruch Sneh. 2007. Genetic diversity, anastomosis groups and virulence of *Rhizoctonia* spp. from strawberry. *European Journal of Plant Pathology* 117 (3):247-265.

Sherwood, R. T. 1970. Physiology of *Rhizoctonia solani*. In *Rhizoctonia solani: Biology and Pathology*, edited by J. R. J. Parmeter. Berkeley: University of California Press

Sneh, Baruch. 1998. Use of non-pathogenic or hypovirulent fungal strains to protect plants against closely related fungal pathogens. *Biotechnology Advances* 16 (1):1-32.

Sneh, Baruch, L. Burpee, and A. Ogoshi. 1991. *Identification of Rhizoctonia species*. St. Paul: APS Press.

Sneh, Baruch, Suha Jajabi-Hare, Stephen Neate, and Gerda Dijst, eds. 1996. *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.* Boston: Kluwer Academic Publishers.

Stalpers, J. A., and T. F. Andersen. 1996. A synopsis of the taxonomy of teleomorphs connected with *Rhizoctonia* s.l. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Stalpers, J. A., T. F. Andersen, and W. Gams. 1998. Two proposals to conserve the names *Rhizoctonia* and *R. solani* (Hyphomycetes). *Taxon* 47 (3):725-726.

Sumner, D. R. 1996. Sclerotia formation by *Rhizoctonia* species and their survival. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control.*, edited by B. Sneh, S. Jajabi-Hare, S. Neate and G. Dijst. Boston: Kluwer Academic Publisher.

Sumner, D. R., and D. K. Bell. 1982a. Crop rotation and yield loss in corn in soil infested with *Rhizoctonia solani* AG-2 and AG-4. *Phytopathology* 72 (3):361-362.

Sumner, D. R., and D. K. Bell. 1982b. Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. *Phytopathology* 72 (1):86-91.

Sweetingham, M. W. 1989. Fungi associated with root and hypocotyl diseases of seedling lupines in Western Australia *Australian Journal of Agricultural Research* 40 (4):781-790.

Swofford, D. L. 2004. *Phylogenetic analysis using parsimony (* and other methods), Version 4.0b10*. Sunderland, Massachusetts: Sinauer Associates.

Tamura, K. , J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599.

Thompson, Julie D., Toby J. Gibson, Frederic Plewniak, Francois Jeanmougin, and Desmond G. Higgins. 1997. The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25 (24):4876-4882.

Toda, T., J. M. Mghalu, A. Priyatmojo, and M. Hyakumachi. 2004. Comparison of sequences for the internal transcribed spacer region in *Rhizoctonia solani* AG 1-ID and other subgroups of AG 1. *Journal of General Plant Pathology* 70:270-272.

Toda, Takeshi, Toshihiro Hayakawa, Joseph Mghalu, Shigeharu Yaguchi, and Mitsuro Hyakumachi. 2007. A new *Rhizoctonia* sp. closely related to *Waitea circinata* causes a new disease of creeping bentgrass. *Journal of General Plant Pathology* 73 (6):379-387.

Tomaso-Peterson, M., and L. E. Trevathan. 2004a. *Rhizoctonia solani* AG-13 isolated from corn in Mississippi. *Plant Disease* 88 (8):908-908.

Tomaso-Peterson, M., and L. E. Trevathan. 2004b. Rhizoctonia solani AG-13 Isolated from Corn in Mississippi.

Tomaso-Peterson, M., and L. E. Trevathan. 2007. Characterization of *Rhizoctonia*-like fungi isolated from agronomic crops and turfgrasses in Mississippi. *Plant Disease* 91 (3):260-265.

Tu, C. C., and J. W. Kimbrough. 1975. Morphology, development, and cytochemistry of hyphae and sclerotia of species in *Rhizoctonia* complex. *Canadian Journal of Botany-Revue Canadienne De Botanique* 53 (20):2282-2296.

Tu, C. C., and J. W. Kimbrough. 1978. Systematics and phylogeny of fungi in the *Rhizoctonia* complex. *Botanical Gazette* 139 (4):454-466.

Tu, C. C., J. W. Kimbrough, and H. C. Aldrich. 1977. Cytology and ultrastructure of *Thanatephorus cucumeris* and related taxa of *Rhizoctonia* complex. *Canadian Journal of Botany-Revue Canadienne De Botanique* 55 (18):2419-2436.

Tu, Chin-Chyu, Ting-Fang Hsieh, and Yih-Chang Chang. 1996. Vegetable diseases incited by *Rhizoctonia* spp. In *Rhizoctonia species: Taxonomy, molecular*

biology, ecology, pathology and disease control., edited by B. Sneh, S. Jabaji-Hare, S. Neate and G. Dijst. Boston Kluwer Academic Publisher.

Vilgalys, R., and M. A. Cubeta. 1994. Molecular systematics and population biology of *Rhizoctonia*. *Annual Review of Phytopathology* 32:135-155.

White, T. J., T. D. Bruns, and L. D. Leach. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A guide to methods and applications.*, edited by M. A. Innis, D. H. Gefland, J. J. Sninsky and T. J. White. San Diego: Academic Press.

Win, H. H., and D. R. Sumner. 1988. Root rot induced in snap bean by *Rhizoctonia solani* AG-4 and AG-2 type 2 in conservation tillage following corn. *Plant Disease* 72 (12):1049-1053.

Yang, C. S., and R. P. Korf. 1985. *Ascorhizoctonia* new-genus and *Complexipes* emend., two genera for anamorphs of species assigned to *Tricharina* discomycetes. *Mycotaxon* 23:457-482.

Yokoyama, K., and A. Ogoshi. 1988. Studies on hyphal anastomosis of *Rhizoctonia solani*. V. Nutritional conditions for anastomosis. *Nippon Kingakukai Kaiho* 29 (2):125-132.